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Velocity Detection and Its Relationship to Filter Feeding in Nymphs of the Mayfly *Ametropus neavei* (Ephemeroptera: Ametropodidae)

BY

Paul J. Schouten

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE.

Department of **ENTOMOLOGY**

Edmonton, Alberta

SPRING 1996

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **VELOCITY DETECTION AND ITS RELATIONSHIP TO FILTER FEEDING IN NYMPHS OF THE MAYFLY *Ametropus neavei* (EPHEMEROPTERA: AMETROPODIDAE)** submitted by **PAUL J. SCHOUTEN** in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE.**

D A Craig

Dr. D. A. Craig (Supervisor)

Jan O Murie

Dr. J. Murie

B. A. Heming

Dr. B. Heming

DATE: 26 - i - 96

ABSTRACT

To determine how nymphs of the mayfly, *Ametropus neavei*, detect water velocity, I examined antennal morphology and did behavioral experiments. Details from SEM micrographs revealed on the antenna external mechanoreceptors which were campaniform sensilla. Histological examination showed internal mechanoreceptors, including Johnston's organ and a new arrangement of scape muscles not reported before.

Results from behavioral experiments on the unique feeding behavior of *A. neavei*, showed that after ablation or immobilization of antennae, detection of water velocity was disrupted, indicating that mechanoreceptors found on the antenna function as water velocity detectors. Detailed descriptions of behavioral changes displayed by *A. neavei* nymphs under different flow conditions show that this insect is an opportunistic feeder, able to adapt to different food resources. Unexpected differences were seen in some behaviors between cohorts of larvae.

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Table of Contents

Chapter	Page
1. Introduction	1
1.1 References	8
2. Functional Morphology of Antennae in Nymphs of the Mayfly *Ametropus neavei* (Ephemeroptera: Ametropodidae)	13
2.1 Introduction	13
2.2 Materials and Methods	16
2.3 Results	19
2.4 Discussion	22
2.5 References	40
3. The Relationship of Feeding Behaviors to Velocity Detection in Nymphs of the Mayfly *Ametropus neavei* (Ephemeroptera: Ametropodidae)	44
3.1 Introduction	44
3.2 Materials and Methods	49
3.3 Results	54
3.3.1 Visual Cues	54
3.3.2 Stereotypic Behaviors	54
3.3.2.1 Settle Down Behavior	54
3.3.2.2 Collector-Gatherer Behavior	55
3.3.2.3 Pit Maintenance Behavior	55
3.3.2.4 Filter Feeding Behavior	55

3.3.2.5 Quantified Behaviors 56
3.3.2 Ablation Experiment 56
3.3.3 Immobilization Experiment 57
3.3.4 Cohort Comparison 58
3.3.5 Percent of Nymphs Filter Feeding 58
3.4 Discussion 60
3.5 References 81

4. General Conclusions 86
4.1 References 89

List of Tables

Table		Page
3-1	Nonparametric multiple comparison test for settle down time in ablation experiment.....	80
3-2	Nonparametric multiple comparison test for settle down time per cohort year.....	80
3-3	Nonparametric multiple comparison test for latency time per cohort year.....	80

List of Figures

Figure	Page
2-1 SEM micrograph overview of head	27
2-2 SEM micrograph of antennal socket	28
2-3 SEM micrograph of antennae	29
2-4 Diagram showing position of sensilla	30
2-5 SEM micrograph showing ring of campaniform sensilla	31
2-6 SEM micrograph showing campaniform sensilla on flagellar article	32
2-7 Reconstruction of scape and pedicel	33
2-8 Sagittal section of scape and pedicel	34
2-9 Longitudinal section of scape and pedicel	35
2-10 Cross section through distal pedicel	36
2-11 Cross section through proximal pedicel	37
2-12 SEM micrograph showing concave cuticle of pedicel	38
2-13 Reconstruction of muscles in scape and pedicel	39
3-1 Diagram of nymph filter feeding with forelegs and antennae extended into flow	68
3-2 Laboratory set up showing flume and video equipment	69
3-3 Extent of flagellar removal in ablation experiment	70

3-4 Extent of antenna glued in immobilization experiment 71
3-5 Settle down behavior of nymphs 72
3-6 Filter feeding behavior with forelegs extended into flow 73
3-7 Mean times of behaviors for November 93 nymphs... 74
3-8 Average time to settle down for nymphs in ablation experiment (velocity 8 cm/s)..... 75
3-9 Average latency time to filter feeding for nymphs in ablation experiment (velocity 18 cm/s)..... 76
3-10 Average latency time to filter feeding for nymphs in immobilization experiment..... 77
3-11 Average settle down time per cohort year..... 78
3-12 Average latency time to filter feeding per cohort year..... 79

List of Abbreviations

abd	abdomen
ant	antennae
apo	apodeme
cam	campaniform sensilla
cco	connective chordotonal organ
che	chemosensilla
con	concave cuticle
dor	dorsal muscle
ext	extrinsic muscle
eye	eye
fla	flagella
for	forelegs
hea	head
int	intrinsic muscle
joh	Johnston's organ
lat	lateral muscle
med	medial muscle
mer	meriston
mou	mouthparts
mul	multiporous plate chemosensilla
ner	nerve
oce	ocelli
ped	pedicel

pit	pit
sca	scape
tho	thorax
tra	trachea
trc	trichoid chemosensilla
tri	trichoid sensilla
ven	ventral muscle

1. Introduction

Current speed is one of the most important physical factors of running water affecting organisms living in rivers and streams (Hynes 1970). Until recently, most studies of lotic systems dealt with the action of water velocity in only general terms (Resh and Rosenberg 1984, Ward 1992). However, Hart and Clark (1996) measured velocity profiles within 2mm of the substrate, a much finer scale than any previous work in the field.

As water flows into different catchment areas of small streams and eventually into large rivers, the volume and velocity of water moving downstream creates many different habitats. In smaller streams, water usually flows with high velocity, leaving only large stable substrates (i.e., large rocks or boulders) not moved by flow or spates. In larger rivers, flow has greater consistency and lowered velocity due to a larger volume of water, allowing smaller substrate sizes (i.e., gravel, sand and silt) to settle out (Newbury and Gaboury 1993, Vogel 1994). Within this range of habitats, current speed determines, for the most part, the occurrence, microdistribution and abundance of species, and affects the structure of communities within lotic systems (Hynes 1970, Resh and Rosenberg 1984, Ward 1992).

An important component of the biology of lotic organisms is their perception of the environment. Invertebrates, specifically insects, are able to respond to a wide variety of environmental stimuli through use of different, specialized sensilla. With these

sensilla, insects are able to detect light, temperature, pressure, motion of media and various chemicals (Autrum 1963, Dethier 1963, Wigglesworth 1964, Gillot 1980, Chapman 1982, McIver 1985, Zacharuk 1985). For aquatic insects, such as nymphs of the mayfly *Ametropus neavei*, it is detection of water movement, or velocity, that is of interest in this study.

A. neavei live as nymphs in large rivers all year, emerging as adults in spring and late summer (Allen and Edmunds 1976, Clifford and Barton 1979). Nymphs inhabit areas of substrate composed of fine sand that is constantly shifted by water velocity (Soluk 1983).

Early observations on feeding behavior of *A. neavei* showed it to belong in the collector-gatherer, functional feeding group (Clifford and Barton 1979, Allen and Edmunds, Jr. 1976, Merrit and Cummins 1978). However, Soluk and Craig (1988) showed this not to be the only feeding behavior exhibited by *A. neavei*. They found nymphs to settle into the sand, with only the head and antennae exposed to water flow. It then dug a pit in front of its mouth parts, creating a solenoidal vortex that increased particle suspension in front of the mouth parts. As water velocity increased beyond 10 cm/s, a unique filter feeding behavior started. Nymphs projected their forelegs into the flow, which directed more water into the pit and allowed the leg hairs to trap more suspended material. This behavior was triggered only when the threshold of 10 cm/s was passed, an indication that nymphs have the ability to detect water velocity.

Water velocity detection should be similar to detection of air speed, only that air is a less dense medium (Vogel 1994). Hollick (1940) demonstrated the importance of antennae as air-speed

indicators for flight in flies. Air flow detection enabled the fly, *Muscina stabulans*, to maintain flight and to change its wing-tip path according to air speed. Antennae have also been shown to function similarly in bees (Heran 1956, Bässler 1958), locusts (Gewecke 1974) and mosquitoes (Roth 1948, McVean 1991).

During flight, both locusts and flies hold their antennae into the wind, with the flagella as stimuli transducers. Gewecke (1974) determined that flagellar deviation is proportional to air speed. Lateral rotation and a steady backwards pressure of the flagella stimulated a single campaniform sensillum located on the pedicel, and Johnston's organ was found to be stimulated by flagellar vibrations. Most sensory cells in this organ respond to vibrational movement, one group detecting lateral rotation and another, medial rotation.

Antennae also enable insects to orient to wind direction. Burkhardt and Gewecke (1966) showed that immobilization and lesioning of flagella in the blow fly, *Calliphora erythrocephala*, caused a curved flight course towards the unaffected side. Orientation to wind has also been shown for a walking dung beetle, *Scaurus dubius*, and a darkling beetle, *Tenebrio molitor*. Linsenmair (1970) described the interaction of paired antennal organs, most likely Johnston's organs, in detecting wind direction and showed that loss of one antenna caused a 50% reduction in turning tendency. After an adequate recovery period, however, a beetle with only one antenna was once again able to determine wind direction and orient itself normally, or turn back to its preferred direction into the wind.

To investigate whether antennae are important in detecting moving media, it is important to have a description of the morphology of the antennae to determine if appropriate sensilla are present. Antennae of most insects consist of two basal segments, the scape and pedicel, followed by an articulated flagellum. The scape contains muscles to move the antenna and the pedicel, sensilla including mechanoreceptors and chemoreceptors. Mechanoreceptors in the pedicel, campaniform sensilla, Johnston's and other chordotonal organs, provide information to the insect about antennal movements. Antennae can move forwards, backwards, side-to-side, rotationally, or in any combination of these movements which are detected (Dethier 1963, Schneider 1964, Gillot 1980, Chapman 1982, McIver 1985, Zacharuk 1985).

Campaniform sensilla are usually located individually on the flagellar articles or in groups on the pedicel. Each consist of thin, dome-shaped cuticle into which is inserted a single sensory neuron. They detect stresses such as shear stress in the plane of the cuticle, and compression of the cuticular dome. Flagellar movement at the joint between the pedicel and flagellum are known to compress the campaniform sensilla and generate information about orientation of the flagellum in relation to the pedicel (Dethier 1963, Schneider 1964, Gillot 1980, Chapman 1982, McIver 1985, Zacharuk 1985).

Johnston's organ is located in the pedicel and generally serves a proprioreceptive function in conjunction with campaniform sensilla. The organ can contain as few as two or three sensory neurons, but as many as 15 000 have been found in the pedicel of male mosquitoes (Belton 1989). These sensory neurons are inserted distally into

cuticle at the articulation between the pedicel and first flagellar article. This arrangement provides information on the position of the antennae in relation to the head and perceives vibrational and rotational movement of the flagellum (Gewecke 1974, Gillot 1980, Chapman 1982, McIver 1985).

Although Johnston's organ is the principal chordotonal organ in the pedicel used for mechanoreception, others have been described. Adults of the locust, *Melanopus mexicanus mexicanus*, has a pedicellar chordotonal organ, and a tip chordotonal organ, in the distal third of the last flagellar article (McFarlane 1953). The connective chordotonal organ generally consists of one to four sensory neurons which are usually attached to cuticle of the pedicel (Debauche 1935, Howse 1968). Other insects have chordotonal organs in the flagella up to and including the fourth article (Eggers 1928).

Since the head and antennae of *A. neavei* nymphs are the only body parts exposed to water flow, these are the most probable sites of sensilla detecting water velocity. While the head may have hair beds, or single trichoid sensilla, similar to other insects, exposed antennae are most likely to receive information about the environment.

Some investigations have shown insects to use antennae to detect various types of water movement, but not water velocity. These investigations determined how hydrodynamic cues, such as surface or pressure waves, are carried through water and detected by insects living on the water surface or in the water column. Aquatic insects studied include back swimmers (Lang 1980), water

striders (Wilcox 1972, Wilcox and Spence 1986), caddisflies (Tachet 1977) and stoneflies (Peckarsky and Wilcox 1989). De Wilde (1941) determined that damaging the pedicel of a whirligig beetle, *Gyrinus* sp., disrupted its ability to orient to disturbances in the water surface film. Disturbances caused the flagella to move and this apparently excited the Johnston's organs (Rudolph 1967).

Hughes (1958) showed that a water beetle, *Dytiscus* sp., holds its antennae forward into the axis of progression, similar to flies and locusts. The path of the beetle deviated in concordance with the degree of bending of antennae. Antennae sensilla of the beetle include Johnston's organ, campaniform sensilla and some external hair plates. More convincing evidence of antennal involvement for course correction was determined when Hughes (1958) sprayed a fine jet of water on one antenna and noted the contralateral hindleg kicked.

The principal objective of this study was to determine if antennae are used for water velocity detection by *A. neavei* nymphs.

Chapter two is a description of the structure and sensilla of the antennae. As expected, there were the normal compliment of both external and internal sensilla. However, the musculature of the scape is unusual and appears to represent a rarely found condition intermediate between very primitive arthropods and higher derived insects.

Chapter three described behavioral experiments which used the unique filter feeding behavior shown by *A. neavei* to determine if the antennae are used for water velocity detection. Antennae

were manipulated by ablation and immobilization with glue, at different lengths, to determine any change in feeding behaviors.

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2. Functional Morphology of the Antennae of Nymphs of the Mayfly *Ametropus neavei* (Ephemeroptera: Ametropodidae)

2.1 Introduction

An important ability for invertebrates living in lotic systems is to perceive their environment. Invertebrates, and more specifically, insects, are able to respond to a wide variety of environmental stimuli through use of different, specialized sensilla. With these sensilla, insects are able to detect light, temperature, pressure, motion of media and various chemicals. Although they are widespread on the insect body, many insects have a concentration of sensilla in the antennae (Schneider 1964, Gillot 1980, Chapman 1982, McIver 1985, Zacharuk 1985).

Nymphs of the mayfly, *Ametropus neavei*, live in fine, sandy substrate of large rivers and bury themselves into the substrate to leave only their head and antennae exposed to water flow (Soluk and Craig 1988). Hence, most information about the environment is probably received by the nymph through its antennae.

The antennae of insects consist of two basal segments, the scape and pedicel, and an articulated flagellum. The scape contains muscles to move the antenna. The pedicel contains sensilla including many mechanoreceptors. The flagellum also has many chemosensilla and mechanosensilla (Schneider 1964, Gillot 1980, Chapman 1982, McIver 1985, Zacharuk 1985).

Campaniform sensilla, Johnston's organ and other chordotonal organs are mechanoreceptors of the pedicel that provide information to an insect about various antennal movements. Solitary campaniform sensilla may also be located on the flagellum (Schneider 1964, Gillot 1980, Chapman 1982, McIver 1985, Zacharuk 1985). Specific mechanoreceptors detect certain movements, such as forward, backward, side-to-side, rotational, or any combination of these, providing complex information about forces moving the antennae (Rudolph 1967, Gewecke 1970, 1974, Kapoor 1985).

Campaniform sensilla consist of areas of thin, dome-shaped cuticle into which is inserted a single sensory neuron. They are used to detect mechanical stresses such as shear in the plane of surface, and compression of the dome. Such sensilla are known to provide information about flagellar orientation relative to the pedicel. Flagellar movements back and forth at the junction of pedicel and flagellum compress each sensillum and stimulate the neuron associated with it (Schneider 1964, Gillot 1980, Chapman 1982, McIver 1985, Zacharuk 1985).

A Johnston's organ is located in the pedicel and usually functions as a proprioreceptor in conjunction with campaniform sensilla. The organ can contain as few as two or three sensory neurons, but as many as 15 000 have been found in male mosquitoes (Belton 1989). These are distally inserted into the cuticle at the articulation of the pedicel and first article of the flagellum. This arrangement provides information on the position of the antennae relative to the head, and more specifically, perceives

of vibrational and rotational movement of the flagellum (Gillot 1980, Chapman 1982, McIver 1985).

Although the Johnston's organ is the principal chordotonal organ used in antennae for mechanoreception, others have been described. A pedicellar chordotonal organ and a tip chordotonal organ, located in the distal third of the last flagellar article, have been described for the locust, *Melanopus mexicanus mexicanus* (McFarlane 1953). Another chordotonal organ is the connective chordotonal organ in insects (Debauche 1935, Howse 1968) and generally consists of one to four sense cells attached at different points to the cuticle of the antennae (Howse 1968). Other insects have chordotonal organs in every segment up to and including the second article of the flagellum (Eggers 1928).

Since *A. neavei* probably detect water velocity with its extended antennae, it was expected that the antennae would have the necessary sensilla, known in other insects to detect air movement. The sensilla were expected to be mechanoreceptors, located internally and externally, and the objective of this study was to describe the sensilla present.

2.2 Materials and Methods

Nymphs of *A. neavei* were collected on May 12, August 4, August 10 and November 3, of 1993 from the Red Deer River at the Emerson Bridge near Brooks, Alberta. A new locality for the species was found on the Pembina River, near Barrhead, Alberta, on August 15, 1994. Nymphs were collected from this site on August 31 and October 26, 1994, and February 14 and May 24, 1995.

Nymphs were collected from the river using a standard D-frame net. This net was scraped along the bottom of the river where water depth was 0.5 to 1 m and the top 3-5 cm of sand removed. After the net was full of sand, excess was washed out and the remainder placed in a small tray to sort out nymphs. Nymphs were placed into 4 l pails containing river water and then into a cooler for transport back to the laboratory.

A paddle-wheel, oval racetrack artificial stream was used to house nymphs in the laboratory (Warren and Davies 1971, Corkum and Pointing 1979, Ciborowski 1983). Water temperature was held at 6°-8° Celsius in a cold room. Water velocity was kept at approximately 15 cm/s. The substrate used was natural sand from the collection sites, with particles mostly 0.25 mm in diameter.

Original water to fill the flume was obtained from the collection sites, that had a pH of 8.0 to 8.5 and conductivity of 250 to 330 µS (microseimens). Water was replaced using artificial stream water, made from a modified protocol (CaCl 1.583g, MgSO4 0.890g, NaHCO3 0.767g, KCl 0.056g to 20l of water) from Bedard *et al* (1992), with a

pH was 8.1 and conductivity 320 µS. Additional river water and natural substrate were added periodically to provide food for nymphs. All nymphs used in microscopic examination were allowed to develop to stage 2 or 3, according to Clifford and Barton (1979), approximately 40 mm in length, since the number of instars are not known for this mayfly.

Specimens for scanning electron microscopy were obtained by removing heads of three nymphs. These were dehydrated to absolute alcohol and critical point dried, mounted on stubs and sputter coated with a fine layer of gold for scanning. The heads were examined and photographed using a JEOL JSM-6301FXV scanning electron microscope.

Specimens for light microscopy (n=12) were fixed in Alcoholic Bouin's. Fixed heads were mounted in a hard (melting point 57° C) paraffin wax (Humason 1967) and sectioned at 6 µm using a Reichert-Jung 2030 microtome. Sections were floated on a warm water bath containing gelatin mountant and retrieved onto glass slides.

After drying, sections were stained with Erhlich's Haematoxylin-Eosin according to Humason (1967) and examined using a Wild M20 compound microscope up to 1000x and with both bright field and phase contrast. Sections were photographed using Kodak Ektar 100 ASA color film and sketched for use in reconstructions using a drawing tube.

Six heads were stained with methylene blue for nerve tissue and whole mounted into Canada Balsam. Six more heads were cleared with 10% KOH solution. and placed on slides with Canada

Balsam. The whole mounts were then examined with normal light and phase contrast to reconstruct organs of the antennae with a standard drawing tube.

2.3 Results

Figure 2-1 shows the head and antennae (ant) of a nymph. Compound eyes (eye) are placed anterolaterally on the head. The ocelli (oce) are located dorsally between the antennae. The mouth parts (mou) and associated hairs are located anteroventrally. Tufted chemosensilla (che) predominate on the head, with only a few trichoid sensilla (tri) at the base of each scape (sca) (Fig. 2-2).

The antennae project anterior from the head and are typical, having a scape (sca), pedicel (ped) and flagellum (fla). Each flagellum comprises of 10 articles in this nymph. The other two heads had, respectively 10 and 11 articles per flagellum. The first proximal article bears a meriston (mer) for all flagella examined (Fig. 2-3).

Many types of chemosensilla are present on the antennae. Trichoid chemosensilla (trc), both uniporous and multiporous are concentrated on the anterofrontal sides of the pedicel and flagellum (Fig. 2-3). Numerous tufted chemosensilla, similar to those on the head, are also present (Fig. 2-6). A series of multiporous plate chemosensilla (mul) (Fig. 2-6) occur along the length of the antennae, with one or two located at the tip and increasing in number down the flagella to the pedicel, but none were found on the scape (Fig. 2-4).

A ring of campaniform sensilla (cam) is located at the distal end of the pedicel where the flagellum inserts (Fig. 2-5) with a few located elsewhere on the pedicel. Single campaniform sensilla are located on flagellar articles (Figs. 2-4, 2-6). Each article may have one, two or three campaniform sensilla, except for the most distal

article which lacks them (Fig. 2-4). The total number of campaniform sensilla for each head was 9, 12, 15, respectively per flagellum.

Histological examination of the antennae shows the expected structures in the scape and pedicel (Fig. 2-7). A main nerve (ner) enters the scape as a large, single tract and divides into two tracts, on opposite sides of the pedicel. Each tract gives rise the Johnston's organ (joh) and divides into a smaller tract continuing up through the pedicel into the flagellum (Fig. 2-8). The Johnston's organ originates at the base of the pedicel, ascends the pedicel parallel the cuticle where it attaches to the distal end of the pedicel into the intersegmental membrane (Figs. 2-7, 2-8, 2-9). Johnston's organ contains up eight chordotonal subunits on each side of the pedicel. A trachea (tra) from the head passes up through the scape and pedicel and on into the flagellum.

An unusual modification of the cuticle of the pedicel is also found (Figs. 2-7, 2-9, 2-11, 2-12). It consists of a concave portion (con) of cuticle that is located anteriorly in the pedicel and is much thinner than elsewhere in the pedicel (Figs. 2-9, 2-11).

A connective chordotonal organ (cco) spans the base of each pedicel (Figs. 2-7, 2-8, 2-9) and attaches proximally to both sides of the pedicel. It ascends the concave cuticle attaching at various points (Figs. 2-9, 2-11).

Two pairs of muscles occur within the scape (Fig. 2-13). An extrinsic pair (ext) originates on the tentorium and inserts at the basal margin of the pedicel on two small apodemes (apo), one ventral and one dorsal (Figs. 2-9, 2-13). A second pair of intrinsic (int)

muscles originates at the base of the scape and inserts on either side of the basal margin of the pedicel (Figs. 2-8, 2-13).

Four muscles insert into the basal margin of the scape (Figs. 2-8, 2-13). One pair is dorsal (dor) and ventral (ven), the other pair is medial (med) and lateral (lat). The lateral/medial pair originate on the head cuticle and the dorsal/ventral on the tentorium.

2.4 Discussion

As expected, sensilla are numerous on the head and antennae of *A. neavei* nymphs. SEM micrographs reveal both chemosensilla and mechanosensilla. Most sensilla on the head are tufted chemosensilla, the only other type, near the antennae, being three trichoid hairs that may be mechanoreceptors (Fig. 2-2).

The antennae bear at least three types of chemosensilla, including tufted, multiporous plate and uniporous trichoid chemosensilla. These sensilla are distributed along the front and inner sides of the antennae, probably to allow more water to be sampled as flow squeezes between the antennae (Vogel 1994).

The ring of campaniform sensilla occurring apically on the pedicel proximal to the joint between the pedicel and flagellum (Fig. 2-5) may be similar to those of locusts, known to give information about flagellar orientation relative to the pedicel. Gewecke (1974) determined flagellar deviation to vary with speed of air flow in locusts. A similar situation may operate in *A. neavei* nymphs where water flow causes flagellar deviation that might compress these sensilla.

Concave cuticle in the pedicel is unique to antennae of *A. neavei*, and has not been described for any other insects (Figs. 2-9, 2-11, 2-12) and may be analogous to the structure of leaf petioles, where the petiole is similarly concave on one surface. Vogel (1992) showed this weakened region to reduce torque stresses acting on the

petiole caused by wind twisting the leaf, and to allow for greater loading of downward force on it without breakage.

Antennae of *A. neavei* nymphs are probably affected the same way by water flow. As water velocity increases, more force is produced and increased stress acts on the antennae. Torque stresses produced by water flowing past the antennae may twist them like a leaf fluttering in the wind. If the concave cuticle is structurally adapted to flow, and like a petiole, reduces the torsion stress and force load caused by water movement, it should be expected to occur in other aquatic insects living in lotic habitats.

Any torsion force caused by twisting of the flagella by water flow is probably detected by campaniform sensilla on the flagellum (Figs. 2-4, 2-6). As the flagellum twists, cuticle will experience torque (Gewecke 1974, Gewecke and Heinzel 1980) and this is known to deform the dome of the campaniform sensilla. Linear arrangement of campaniform sensilla along the inner side of each flagellum may allow for sequential stimulation of sensilla as more torque increases twisting of flagellar cuticle.

Vibrational movement (pers. obs.) of the antennae may also help *A. neavei* orient itself in relation to water flow. The flagella show similar movement as described for antennae of beetles (Linsenmair 1970), locusts and flies (Burkhardt and Gewecke 1966, Gewecke 1974) when moved by air currents and to those of water beetles (Hughes 1958) when moved by water currents. Rudolph (1967) showed minor movement of water to move the antennae of the water beetle, *Gyrinus substiatus* Steph. enabling it to orient itself to the disturbance. In general, as flagella are moved about their

longitudinal axis to the right or left, insects detect direction of air or water flow past their antennae.

Histological examination of antennae of *A. neavei* nymphs also revealed a number of internal sensilla probably involved in mechanoreception. The pedicel contains a Johnston's organ and a connective chordotonal organ (Figs. 2-7, 2-9). Arrangement of components of the Johnston's organ within the pedicel is similar to that of caddisfly larvae (Debauche 1935), blow flies (Gewecke 1974) and locusts (McFarlane 1953). Use of antennae to sense air flow and control flight has been established in the blow fly, *Calliphora erythrocephala*, through behavioral experiments (Gewecke 1974). The connective chordotonal organ (Bode 1986, Howse 1968, Schmidt 1974) in the pedicel (Figs. 2-9, 2-11), is probably used to detect flagellar movement, most likely torque (McVean 1991) applied to the pedicel by flagellar rotation.

Besides responding to air speed, sensilla in antennae may detect airborne sound. In some flies, sensitivity of Johnston's organ to sound frequencies of up to 500 Hz has been found (Burkhardt and Gewecke 1966). Roth (1948) showed that male of *Aedes aegypti* respond to frequencies of up to 800 Hz, the wing beat frequency of female mosquitoes. Structure of the antennae has been adapted for this purpose through expansion of the pedicel to house a large, elaborate Johnston's organ registering flagellar vibration caused by sound waves. A Johnston's organ in each pedicel should enable *A. neavei* nymphs to perceive similar vibrations due to water flow.

Movement of flagella, whether vibrational, rotational, flexion and extension, or any combination of these, are all passive, caused by

flow forces. However, not all movement of antennae are passive. The scape of *A. neavei* antennae, is well supplied with intrinsic and extrinsic muscles (Figs. 2-8, 2-9, 2-13) moving the scape and pedicel.

In primitive insects, such as thysanurans, four extrinsic muscles move the scape (Chaudonneret 1950) and is also found in the adult lepidopteran, *Micropteryx* (Hannemann 1956). These muscles originate on the tentorium and head capsule wall and insert along into the basal margin of the scape. These are arranged as two functional groups, one acting as levators and the other as depressors (Imms 1939, Matsuda 1965, Snodgrass 1935).

A primitive condition, hypothesized by Matsuda (1965), has a scape with four extrinsic muscles inserted on the basal margin, two dorsal (51, 52) and two ventral (53, 54) and was found in *Micropteryx* (Hannemann 1956). He further postulated that primitive antennae scape has four intrinsic muscles originate at the base and insert to the base of the pedicel.

In neuropteroid insects, there is a trend towards reduction of muscles of the scape. The number of muscles inserting into the basal margin of the pedicel is reduced to two intrinsic muscles in some insects, with four extrinsic muscles inserting into the basal margin of the scape (Matsuda 1965). In thrips, further reduction of the muscles occurs with the scape having only two intrinsic muscle and two extrinsic muscles (Heming 1975). These still function generally as levators and depressors of the antennae. In lepidopterous caterpillars, there is reduction to one muscle acting as a retractor for the whole antenna (Matsuda 1965).

In *A. neavei* nymphs, muscle disposition in the scape is similar to the primitive condition hypothesized by Matsuda (1965). There are four extrinsic muscle inserted at the basal margin of the scape and function as levators and depressors respectively (Fig. 2-13). However it is not clear whether these extrinsic muscles of *A. neavei* function in manner analogous to Matsuda's description.

Two intrinsic scape muscles are inserted lateral and medial into the pedicellar basal margin, and probably function as stabilizers of lateral motion caused by water motion acting on the antennae. However, the other pair of muscles are extrinsic, where Matsuda (1965) hypothesized another intrinsic pair. This has not been reported before. This pair of muscles originate on the tentorium and insert into cuticular apodemes of the pedicel (Figs. 2-9, 2-13). They are dorsal and ventral in orientation and probably function as levators and depressors.

This unusual and unique arrangement of muscles should not be unexpected given the primitive phylogenetic status Ephemeropterans are generally assigned. Within mayflies, the family Ametropodidae, to which *Ametropus neavei* is a member, is considered among the more primitive of extant families (Gillot 1980, Chapman 1982, Hubbard 1990). However, that assertion will need to be tested by examining antennal musculature of taxa related to *A. neavei*, because musculature might be an adaptation to flow forces acting on the antennae of nymphs.

Fig. 2-1 SEM micrograph of head showing antennae (ant), compound eye (eye), ocelli (oce), mouthparts (mou) and chemosensilla (che). Scale bar = 2 mm.

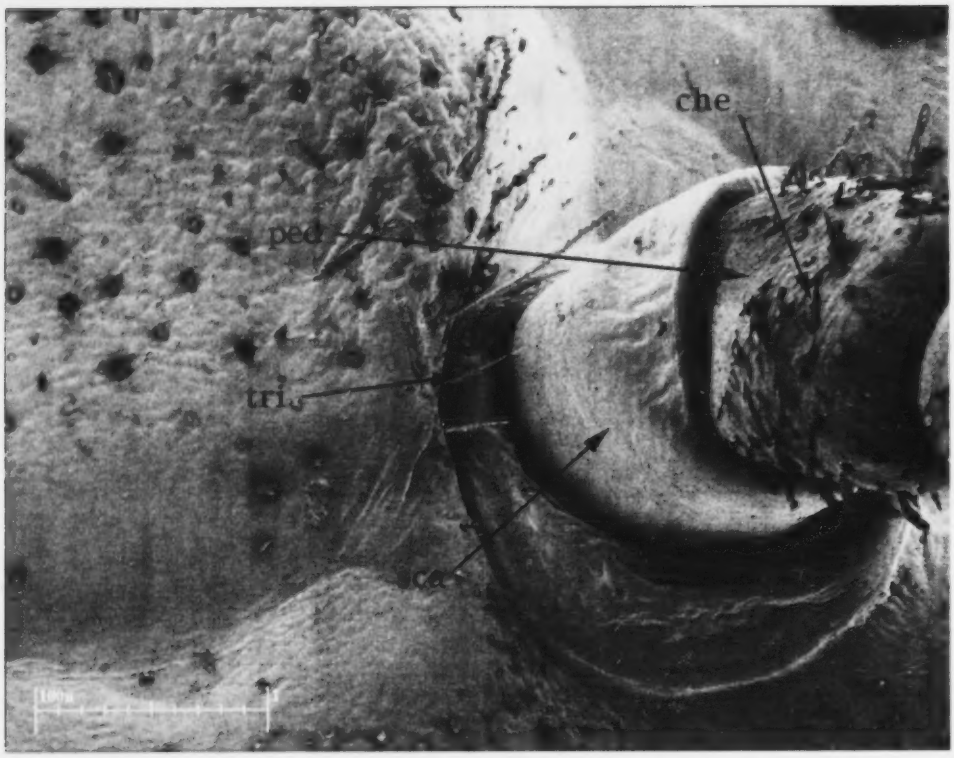

Fig. 2-2 SEM micrograph of antennal socket showing trichoid sensilla (tri), scape (sca), pedicel (ped) and chemosensilla (che). Scale bar = 1 mm.

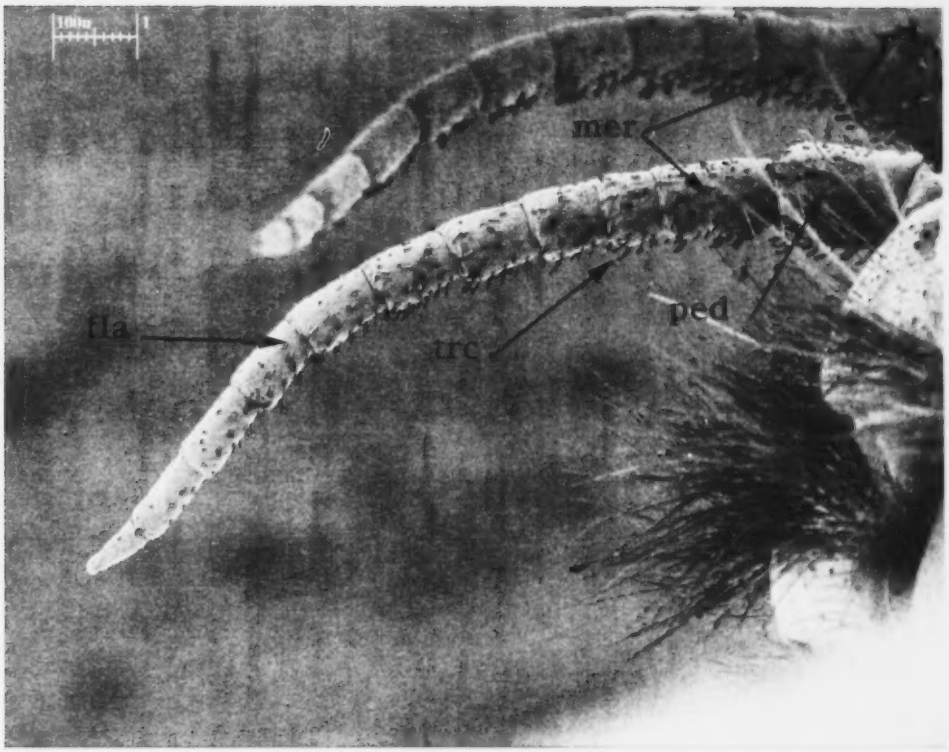

Fig. 2-3 SEM micrograph of antennae showing the flagellum (fla), trichoid chemosensilla (trc), meriston (mer) and pedicel (ped). Scale bar = 1 mm.

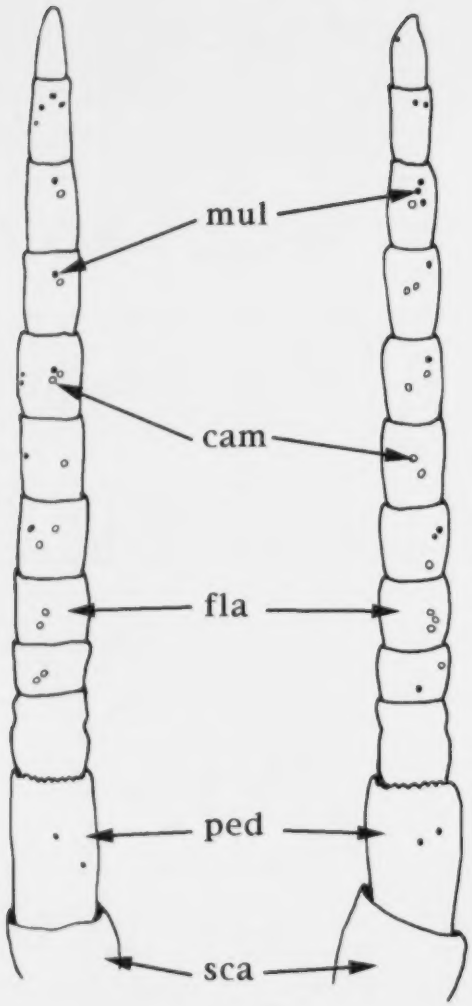

Fig. 2-4 Diagram showing position of multiporous plate chemosensilla (mul, ●) and campaniform sensilla (cam, o) on the scape (sca), pedicel (ped) and flagellum (fla). Dorsal aspect, not to scale.

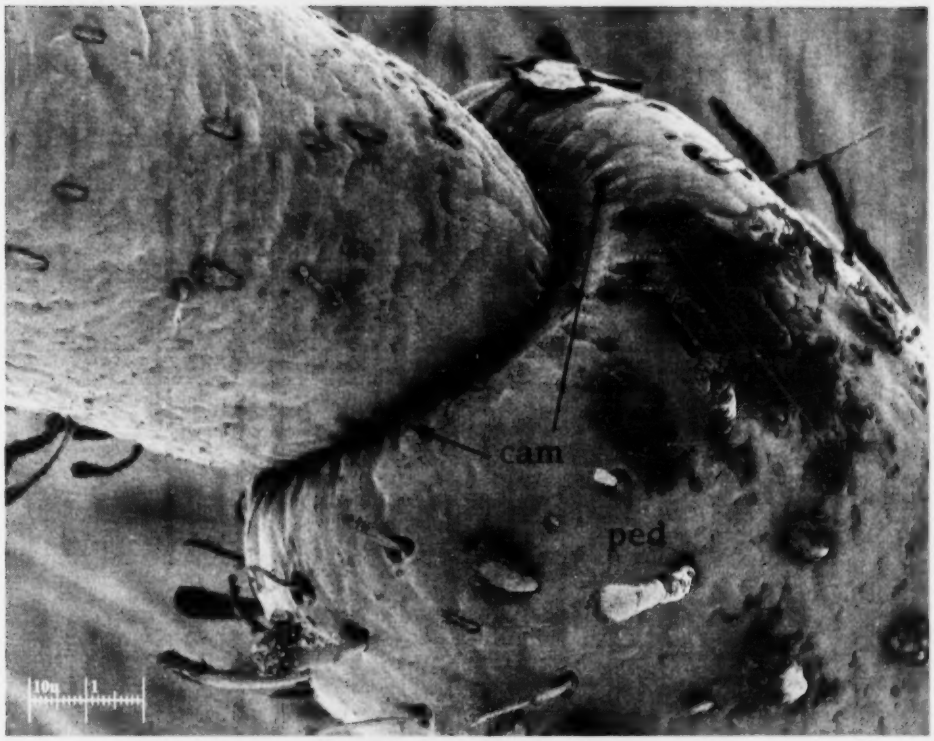

Fig. 2-5 SEM micrograph showing the ring of campaniform sensilla (cam) at the apex of the pedicel (ped). Scale bar = 200μm.

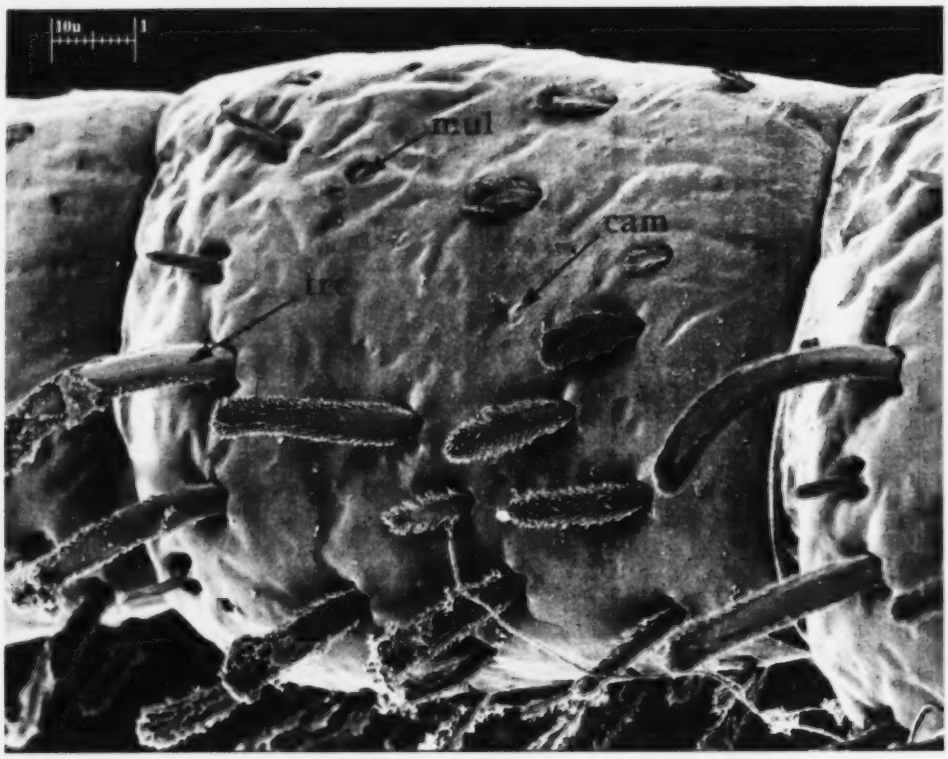

Fig. 2-6 SEM micrograph showing campaniform sensilla (cam), multiporous plate chemosensilla (mul) and trichoid chemosensilla (trc) on a flagellar article. Scale bar = 100 μm.

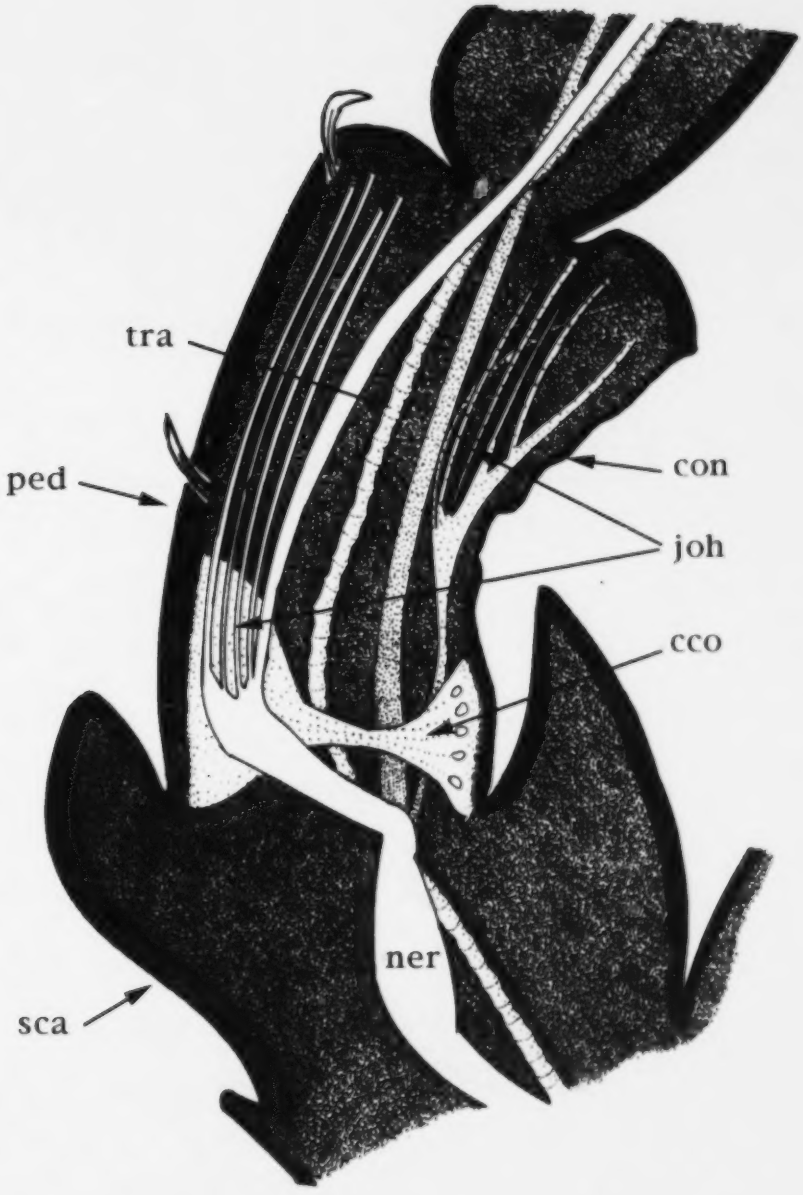

Fig. 2-7 Reconstruction of scape (sca) and pedicel (ped) showing main nerve (ner), trachea (tra), connective chordotonal organ (cco), concave cuticle (con) and Johnston's organ (joh). Not to scale.

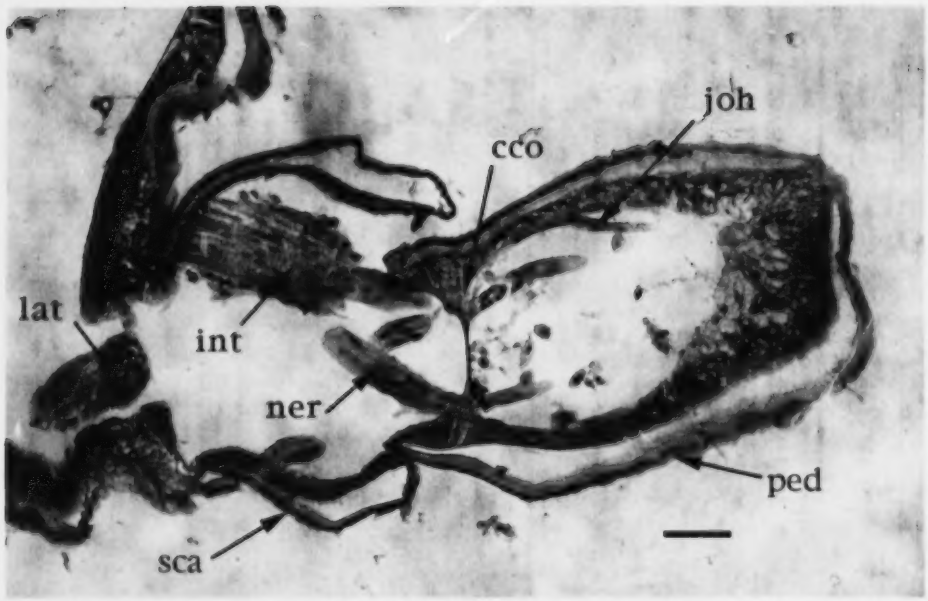

Fig. 2-8 Sagittal section of scape (sca) and pedicel (ped) showing the nerve (ner), Johnston's organ (joh), connective chordotonal organ (cco), intrinsic muscle (int) and lateral muscle (lat). Scale bar = 18 μm.

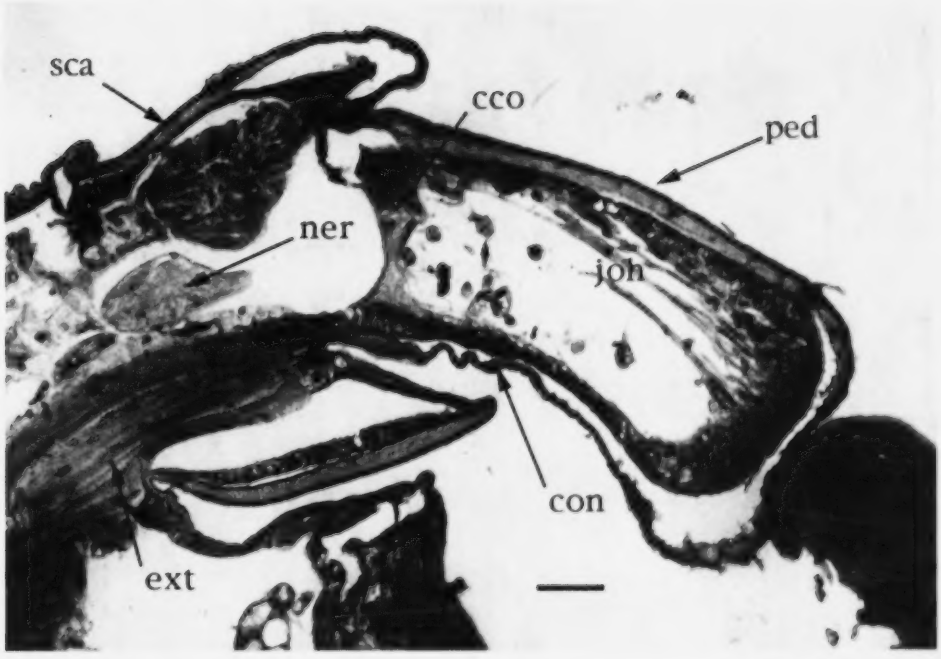

Fig. 2-9 Longitudinal section of scape (sca) and pedicel (ped) showing the nerve (ner), Johnston's organ (joh), connective chordotonal organ (cco), concave cuticle (con), and extrinsic muscle (ext). Scale bar = 18 μm.

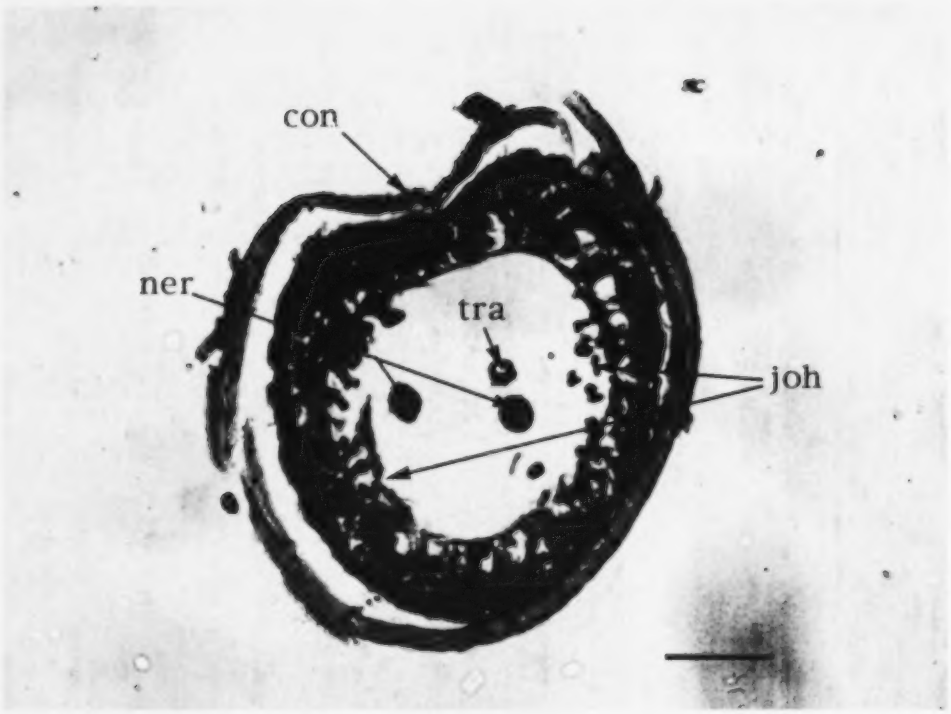

Fig. 2-10 Cross section through distal pedicel showing Johnston's organ (joh), nerves (ner), concave cuticle (con) and trachea (tra). Scale bar = 30 μm.

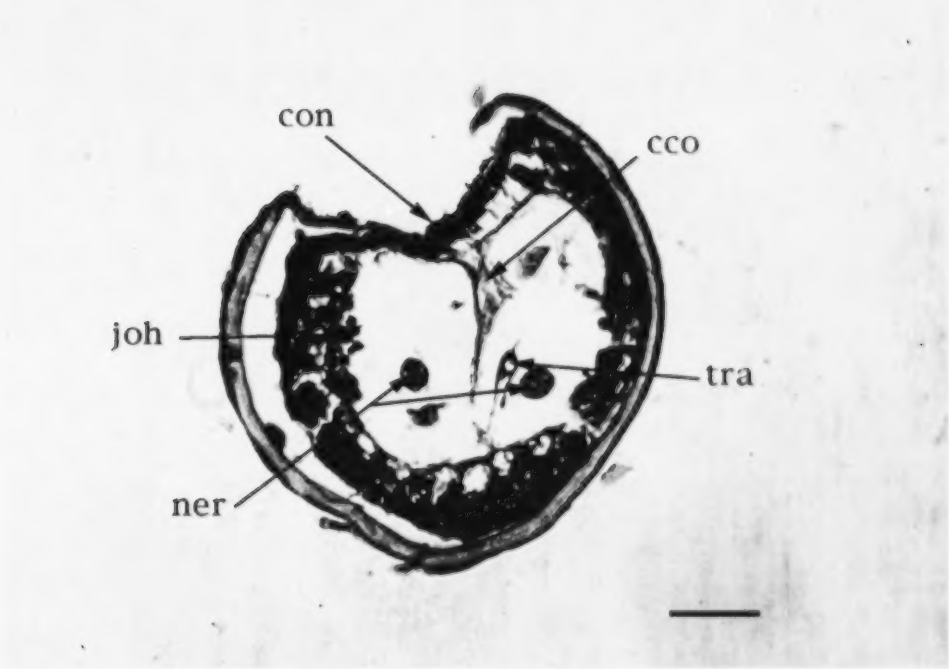

Fig. 2-11 Cross section through proximal pedicel showing connective chordotonal organ (cco), Johnston's organ (joh) nerves (ner), concave cuticle (con) and trachea (tra). Scale bar = 30 μm.

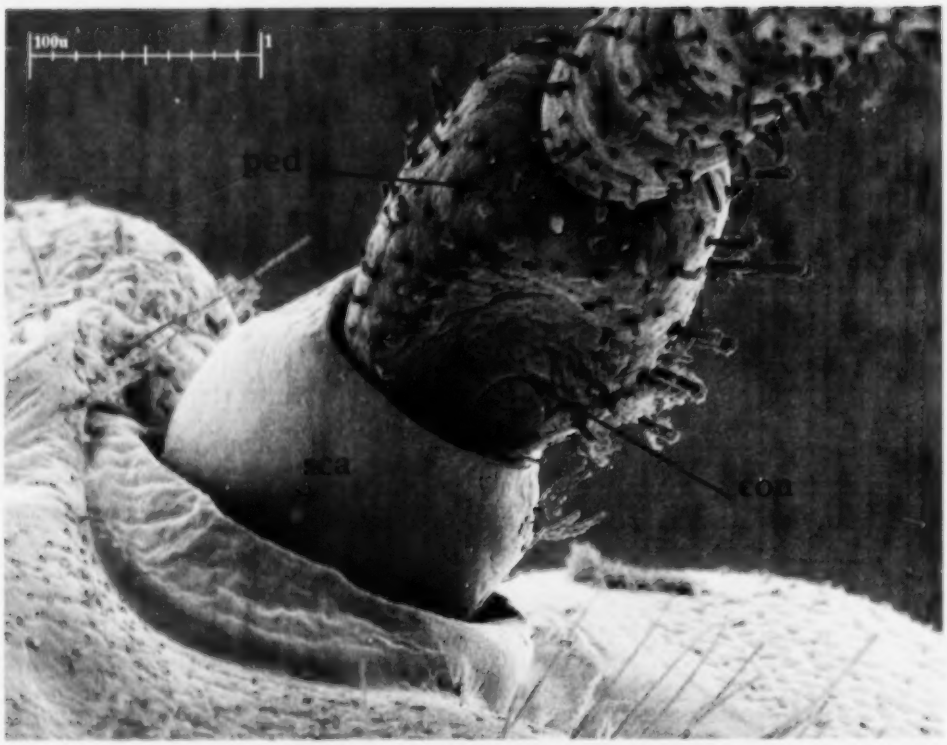

Fig. 2-12 SEM micrograph of anteroventral side of pedicel (ped) and scape (sca) showing concave cuticle (con). Scale bar = 1 mm.

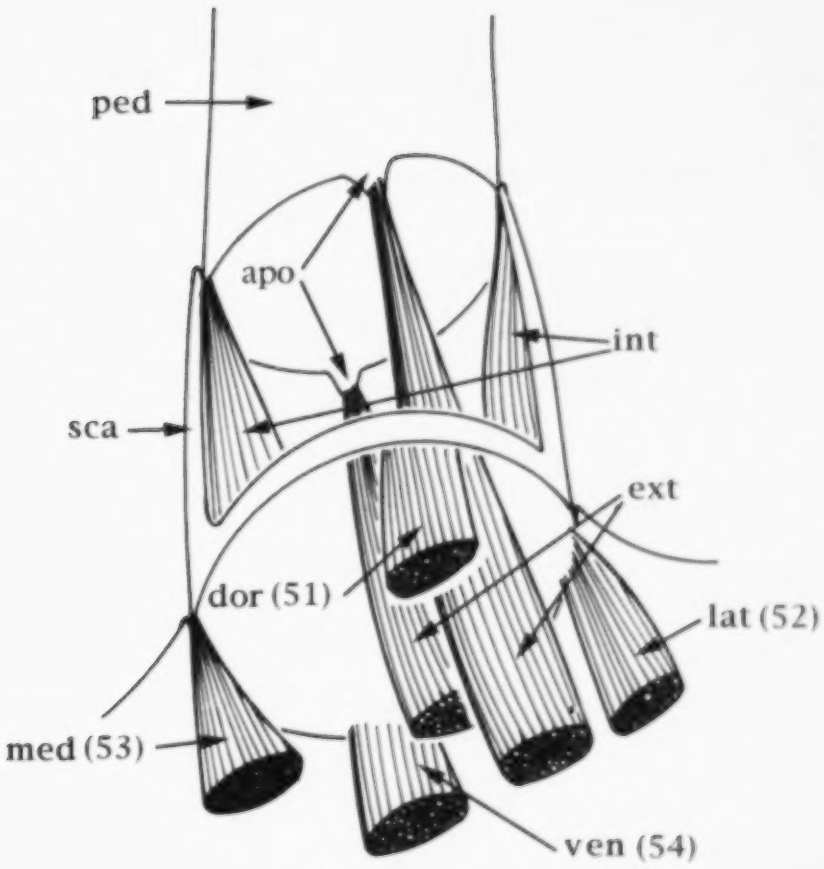

Fig. 2-13 Reconstruction of muscles in the scape (sca) and pedicel (ped) with cuticular apodemes (apo). Two extrinsic (ext) and two intrinsic (int) muscles insert on the pedicel. Four extrinsic muscles, dorsal (dor), ventral (ven), lateral (lat) and medial (med), insert on the basal margin of the scape. Numbers in parantheses refer to homologous muscles in Matsuda (1965). Not to scale.

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3. The Relationship of Feeding Behaviors to Velocity Detection in Nymphs of the Mayfly *Ametropus neavei* (Ephemeroptera: Ametropodidae).

3.1 Introduction

Feeding behaviors of aquatic insects are important in understanding community dynamics and predicting ecological relationships (Cummins 1973, Vannote *et al*, 1980, McShaffrey and McCafferty 1986). Early work has often been speculative and anecdotal. Conclusions regarding the feeding behavior of one species have often been based on casual observation of another species with similar morphology. However, recent work on benthic insects has placed greater emphasis on increased rigor in observing feeding behavior (Keltner and McCafferty 1986, McShaffrey and McCafferty 1986 1988, 1990).

For example, original descriptions of the feeding behavior of *A. neavei* based on casual observation of nymphs under non-flow conditions, led to the conclusion that this insect feeds as a collector-gatherer (Merrit and Cummins 1978, Clifford and Barton 1979). However, *A. neavei* nymphs live in constantly flowing conditions in large rivers (Allen and Edmunds 1976, Clifford and Barton 1979), and inhabit areas of substrate composed of fine sand that constantly shifts due to water current (Soluk 1983). Soluk and Craig (1988) showed in laboratory flumes that with variable flow conditions, the nymphs would filter feed.

Soluk and Craig (1988) described how a nymph settled into the sand and dug a pit in front of its mouth parts (Fig. 3-1). Once the water velocity increased above 10 cm/s, the nymphs projected their forelegs (for) into the flow to filter feed (Fig. 3-1). Water flow was deflected into the pit and created a solenoidal vortex that increased particle suspension in front of the mouth parts.

This raised the question of how nymphs change feeding behaviors under different flow conditions? Soluk and Craig (1988) showed that a water velocity threshold must be passed before filter feeding began. This indicated that nymphs are able to detect changes in water velocity. Since only the head (hea) and antennae (ant) are exposed to water flow after the nymph burrows into the substrate, it is likely that these structures are involved in its ability to detect water velocity (Fig. 3-1). Presumably, sensilla present on the antennae function to detect water velocity (Chapter 1).

Other work has shown aquatic insects to use antennae to detect various hydrodynamic factors, though not water velocity. These investigations include, surface wave detection by water striders (Wilcox 1972, Wilcox and Spence 1986) and back swimmers (Lang 1980) caused by prey moving on the water surface. Larvae stoneflies (Peckarsky and Wilcox 1989) and caddisflies (Tachet 1977) locate prey by detecting pressure waves and vibrations transmitted through water.

Specifically, de Wilde (1941) determined that damaging the pedicels of a whirligig beetle, *Gyrinus* sp. antennae disrupted the insect's ability to orient to disturbances of the water surface film. Such disturbances caused the flagella to move and Rudolph (1967)

concluded that these movements stimulate the Johnston's organs. Hughes (1958) showed that a water beetle, *Dytiscus* sp., held its antennae forward into the axis of progression. The path of the beetle deviated according to the degree of flexion of the antennae. Also, a fine jet of water sprayed on one antenna caused the contralateral hindleg to kick, more evidence of antennal involvement in course correction. Hughes (1958) also described sensilla of the antennae including Johnston's organs, campaniform sensilla and external hair plates.

Detection of water velocity should be similar to detecting air speed, since air is simply a less dense medium (Vogel 1994). Hollick (1940) demonstrated the importance of antennae as air-speed indicators for flight in flies. Air flow detected by its antennae enabled a *Muscina stabulans* fly to maintain flight and to change its wing-tip path according to air speed. Antennae have been shown to function as similar air-speed indicators in bees (Heran 1956, Bässler 1958), locusts (Gewecke 1974) and mosquitoes (Roth 1948, McVean 1991).

During flight, both locusts and flies hold their antennae forward into the wind, using the flagella to receive stimuli. Gewecke (1974) determined that flagellar deviation was proportional to air speed. Lateral rotation and steady, backwards pressure on the flagella stimulate a single campaniform sensillum located on the pedicel of each antenna. Johnston's organs were also found to be stimulated by flagellar vibration. Most sensory cells in this organ respond to vibrational movement, one group detected lateral rotation and another, medial rotation.

Antennae are also important for insect orientation to wind direction. Burkhardt and Gewecke (1966) showed immobilization and lesioning of flagella in the blow fly, *Calliphora erythrocephala*, to cause its flight course to alter towards the unaffected side. Linsenmair (1970) described orientation to wind in the walking dung beetle, *Scaurus dubius*, and in a darkling beetle, *Tenebrio molitor*. He showed paired antennal sensilla most likely Johnston's organs, to detect wind direction. Loss of an antenna caused a 50% reduction in the turning tendency for these beetles. However, after an adequate recovery period, a beetle with only one antenna was again able to determine wind direction and orient itself, or to turn back, to its preferred direction into the wind.

Since insect antennae are known to detect air and water movements, and since in *A. neavei* nymphs it is only antennae that extend into the water column when the insect is not filter feeding, it is not unreasonable to assume that antennae are the main detectors of water velocity. In this chapter I test the hypothesis that filter feeding behavior of *A. neavei* nymphs is affected by experimental modification of the antennae.

To assess effects of experimental manipulation upon an organism's behavior, it is important to understand behavior under conditions as near to natural as possible. Since nymphs of *A. neavei* live in large rivers, they are constantly exposed to water flow. Therefore, nymphs were studied under conditions of flow and non-flow. I used of a small flume (Lacoursière and Craig 1990), in which water velocity can be controlled and videotaped behavior of *A. neavei* nymphs exposed to different water velocities. From the

videotapes, a basic set of stereotypic behaviors were determined and used as a basis to test if experimentation on other nymphs affected their ability to detect water velocity.

3.2 Materials and Methods

Nymphs of *A. neavei* were collected on November 3, 1993, at Emerson Bridge on the Red Deer River near Brooks, Alberta. A new locality for this species was found on the Pembina River, near Barrhead, Alberta, on August 15, 1994. Nymphs were collected from this site on August 31 and October 26, 1994, and on February 14, May 24, August 28, and October 15, 1995.

Nymphs were collected using a standard D-frame net by scraping it along the substrate at water depths of 0.50 to 1.00 m. The top 2-3 cm of sand was removed for a length of about one meter. After the net was full of sand, the excess was washed out and the remainder placed in a small tray to sort nymphs. Nymphs were placed into 4 l pails and then into a cooler for transport back to the laboratory.

A paddle-wheel, oval racetrack, artificial stream was used to house nymphs in the laboratory (Warren and Davies 1971, Corkum and Pointing 1979, Ciborowski 1983). Water temperature was held at 6°-8° C in a cold room while velocity was maintained at 15.0 cm/s. The substrate used was natural sand from collection sites, fine particles mostly 0.25-0.50 mm in size.

Original water used to fill the flume was from the collection sites, that had a pH of 8.0 to 8.5 and conductivity of 250 to 330 µS (microseimens). Water was replaced with artificial stream water, made from a modified protocol (CaCl 1.583g, MgSO4 0.890g, NaHCO3 0.767g, KCl 0.056g to 20l of water) from Bedard *et al* (1992), with a

pH of 8.1 and conductivity of 320 µS. Additional river water and natural substrate were added periodically to provide food for nymphs. All nymphs used in quantifying behaviors and in experiments were allowed to develop to stage 2 or 3 according to Clifford and Barton (1979), approximately 40 mm in length, since the number of instars are not known for this mayfly.

Experiments were done in a small flume as described by Lacoursière and Craig (1990) (Figure 3-2). A diffuser was added to condition flow by equalizing flow in top portion of the flume to the bottom. Water used in this flume was the same artificial stream water used in the rearing flume. Sand, 0.25-0.50 mm in diameter, was used as substrate. A JVC Saticon video camera with a Kipar Zoomit camera lens with x4 magnification was used for filming. The lens was fitted to the video camera using a C adapter along with a 4 cm extension tube to increase magnification up to x10. The camera was mounted on a wheeled tripod so it could be easily moved. A Panasonic AG-6200 VHS video cassette recorder (VCR) was used to record all observations made in the flume. Video output from both the VCR and the camera was monitored on a Sony Trinitron 14 " monitor. Lighting was provided by a Wild high intensity lamp. Water velocity was measured using a Stream Flo 422 meter with a low velocity probe. Duration of events was determined using a Sargent-Welch stopwatch and the timing clock on the VCR.

To describe behavior of *Ametropus neavei*, 50 nymphs were observed for common behaviors and response to visual cues (see 3.3.1) occurring at non-flow (0 cm/s), low flow (8 cm/s) and high flow (12 and 18 cm/s) water velocities. Two substrate types were

used 1) natural river sand that contained organic material and 2) washed sand. This was done to determine if visual cues (dislodged organic material in the water column) were used by nymphs to detect water velocity.

Twenty-five nymphs from the Nov, 1993 cohort were observed and videotaped under various flow conditions to describe and quantify behavior (see 3.3.2) to be used in experimental treatment. All nymphs were introduced to the flume at a water velocity of 8.0 cm/s to observe their settle down behavior (Soluk and Craig 1988)(see 3.3.2.1). An average time for this behavior (see 3.3.2.5) was determined by measuring the period from introduction to when they had finished excavating their pits. Nymphs were then exposed to random water velocities of 0.0, 8.0, 12.0 and 18.0 cm/s. Velocities of 0.0 and 8.0 cm/s were chosen to obtain a complete description of collector-gatherer behavior (Clifford and Barton 1979) (see 3.3.2.2). Observations at velocities of 12.0 and 18.0 cm/s were made to determine average latency time (see 3.3.2.5) to the start of filter feeding behavior (Soluk and Craig 1988) (see 3.3.2.4). These velocities were chosen based on the assumption that less antennal surface area might require higher velocity to initiate filter feeding behavior (see 3.3.3). Videotapes were examined to determine if any other stereotypic behaviors were exhibited by nymphs.

The first experiment (see 3.3.3) considered the effects of flagellar ablation at various lengths on ability to detect velocity. Treatments (Fig. 3-3) involved the ablation of flagella of the a) distal one third (0.3AB), b) distal two thirds (0.6AB) and c) complete

removal (1AB). Ten nymphs were used for each treatment and ten unmodified nymphs for the experimental control.

Nymphs used were from the Aug, 1994 cohort. All nymphs were removed from the rearing tank and cooled on ice for two hours to slow them down for ease of handling. Antennae of nymphs were cut to predetermined lengths in a random design. After ablation, nymphs were allowed to adjust to room temperature for 60 minutes. Nymphs were introduced to the flume at a water velocity of 8.0 cm/s and allowed to settle down into the substrate. This period was recorded. After completing the settle down behavior, nymphs from each treatment were exposed to water velocities of 0.0, 8.0, 12.0 and 18.0 cm/s in a random design and all resulting behaviors videotaped for further analysis. After each treatment, nymphs were preserved in 70% alcohol.

The second experiment (see 3.3.4) involved the use of glue to immobilize the antennae. In one treatment, glue was applied only to the scape and pedicel allowing free movement of the flagella. In the second treatment, portions of antennae were immobilized with glue as shown in Figure 3-4. Exact amounts of glue applied to each nymph could not be determined until after the experiment by examination of preserved nymphs which resulted in unequal sample sizes.

Thirty-nine nymphs from the Oct, 1995 cohort, were used for this experiment. These were taken from the rearing flume and cooled as above. A fine sable hair attached to the tip of a dissection probe was used to apply a small amount of glue (Loctite 454 gel; active ingredient cyanoacrlyate ester) to each antennae. After

gluing, nymphs were placed in a beaker containing 50 ml of cooled water and allowed to warm to room temperature for 60 minutes. Nymphs were then introduced to the flume at a water velocity of 8.0 cm/s and allowed to settle down into the substrate. They were then exposed to a water velocity of 12.0 cm/s and all behaviors videotaped. All nymphs used in the treatment were preserved in 70 % alcohol for later examination.

Preserved nymphs from both experiments were examined using a dissecting microscope and checked to see if they were pharate (*i.e.*, preparing to molt). Any pharate nymphs or had incorrectly glued antennae, were removed from the analysis.

3.3 Results

3.3.1 Visual Cues

Nymphs (n=20) from August 1993 observed in natural water and substrate performed filter feeding behavior once the velocity threshold of 10 cm/s was reached. These nymphs, introduced to clean water and substrate that had no organic material or particles to be suspended in the water column by flow, again performed filter feeding behavior once the velocity threshold was reached.

3.3.2 Stereotypic Behaviors

3.3.2.1 Settle Down Behavior

Nymphs were observed under both low-flow (velocity <8.0 cm/s) and non-flow water conditions and were found to settle down as described by Soluk and Craig (1988) first orienting themselves forward into the flow as they swam down to the substrate. Nymphs in non-flow conditions showed no oriented swimming to the substrate and buried themselves in an apparently random fashion. Once on the sand, nymphs buried themselves under a thin layer of sand, covering the abdomen (abd) and thorax (tho) by shifting their bodies side-to-side while pulling themselves into the substrate with their legs (Fig. 3-5a-c). When buried in the sand, nymphs used their forelegs to excavate a shallow pit (pit) in front of the head (hea). Vertical abdominal undulations by nymphs were continued throughout this process.

3.3.2.2 Collector-Gatherer Behavior

Nymphs of *A. neavei* exhibited a collector-gatherer behavior described in earlier studies (Merrit and Cummins 1978, Clifford and Barton 1979). The forelegs were used to sweep particles of sand and detritus into the pit and towards the mouth parts. Individual particles were rolled with the labial and maxillary palps, while the mandibles scraped off adhering organic material. Upon completion, each particle was discarded by the mouth parts and swept out of the pit by the forelegs. If nymphs were kept in low or non-flow conditions for extended periods of time, furrows were made in the sand as they moved through the substrate in search of food.

3.3.2.3 Pit Maintenance Behavior

Nymphs performed pit maintenance behavior under both flow and non-flow conditions. While exposed to flow, water would move substrate and fill the pit. Nymphs cleared the pit by flexing their forelegs into the center of the pit and sweeping them up and backwards, to move out accumulated debris. This behavior occurred frequently in nymphs exposed to high water flow and less frequently in nymphs in low-flow and non-flow conditions.

3.3.2.4 Filter Feeding Behavior

Filter feeding was observed after nymphs had settled down and constructed a pit. It was triggered when a velocity threshold of 10.0 cm/s was surpassed at which point, the nymphs extended their forelegs (for) into the flow (Fig. 3-6). While so extended, leg hairs

would become clogged with organic particles. Nymphs then retracted each leg out of the flow down to the mouth parts where the leg hairs were cleaned. Antennae (ant) were also cleaned during filter feeding by combing the forelegs along the length of each antenna, starting from the base and moving to the tip, to remove particles adhering to antennal hairs.

3.3.2.5 Quantified Behaviors

Nymphs from the November 1993 cohort were used to determine a length of time to videotape settle down behavior and the latency time to filter feeding behavior. A total of 25 nymphs were videotaped, but 11 were discarded due to poor visibility, being damaged or in a pharate stage.

The mean time to settle down and latency time for nymphs are shown in Figure 3-7. The range for settle down time was 98 to 240 seconds and was 9 to 411 seconds for latency time. The subsequent observation period used for each behavior was 300 seconds.

3.3.3 Ablation Experiment

Filter feeding behavior was observed and videotaped for control nymphs at water velocities of 12.0 and 18.0 cm/s. Filter feeding was observed and videotaped for the 0.3AB treatment at a water velocity of 18.0 cm/s. The 0.6AB treatment showed no filter feeding at any velocity (0.0, 8.0, 12.0 and 18.0 cm/s). Likewise for the 1AB treatment, with one exception, a nymph started to filter feed after the settle down behavior at 8.0 cm/s. It began to filter feed at

a velocity of 12.0 cm/s and continued to filter feed at velocities of 18.0 cm/s and 0.0 cm/s.

The mean settle down times for the control and treatment nymphs are shown in Figure 3-8. A Kruskal-Wallis test and showed a difference (N=38, H_c= 13.738, $X^2_{0.05,3}$= 7.815) within the group. A Nonparametric Multiple Comparison test (Zar 1984) showed that differences within the group were between the control and the 0.3AB treatment (Table 3-1). A difference between the control and both 0.6AB and 1AB treatments was not found. There was no difference between 0.3AB, 0.6AB and 1AB treatments.

A Mann-Whitney test was done between mean latency times to filter feeding behavior for control and 0.3AB treatment at a water velocity of 18.0 cm/s and a difference was found between the two means for latency time (n_1= 8, n_2= 5, U=0, U'= 45, $U'_{0.05,(2),5,8}$= 34) (Fig. 3-9).

3.3.4 Immobilization Experiment

Settle down times for control nymphs and each treatment were all within 300 seconds, as determined by the mean time for the cohort from November, 1993, but no measurements of this time were recorded for this experiment.

Filter feeding behavior was observed in control nymphs, in those with the scape and pedicel glued with two free flagella (G2F) and one free flagellum (G1F). No filter feeding was observed for the treatment where the scape, pedicel and both flagella glued and immobilized (G0F). Mean latency times for the control, G2F and G1F

nymphs, are shown in Figure 3-10. A Kruskal-Wallis test showed no difference (N= 39, H_c= 1.0138, $X^2_{0.05,2}$= 5.991) between these means.

3.3.5 Cohort Comparison

Means of settle down and latency time for each cohort were compared against each other to test for differences among cohorts. A Kruskal-Wallis test on means for settle down time (Fig. 3-11) showed a difference among the cohorts (N= 44, H_c=15.3137, $X^2_{0.05,2}$= 5.991). A Nonparametric Comparison test (Zar 1984) showed that the difference was between November, 1993 and August, 1994, between November, 1993 and October, 1995, but not between August, 1994 and October, 1995 (Table 3-2).

A Kruskal-Wallis test also showed a difference (N=41, H_c= 13.7591, $X^2_{0.05,2}$= 5.991) among cohorts for mean latency times (Fig. 3-12). A Nonparametric Comparison test (Zar 1984) showed differences between November, 1993 and October, 1995 and between August, 1994 and October, 1995, but no difference between November, 1993 and August, 1994 (Table 3-3).

3.3.6 Percent of Nymphs Filter Feeding

Totals for each percentage derived, have been corrected for nymphs that were excluded for being pharate, damaged or buried under sand dunes at higher water velocities. 100% of nymphs fed for each cohort (Nov 93, n=14; Aug 94, n=8; Oct 95, n=19). 50% of nymphs in the 0.3AB treatment (n=8) and 100% of control nymphs (n=10) performed filter feeding behavior in the ablation experiment. Nymphs in treatments of G1F (n=18) and G2F (n=9) had 66% and 83%

filter feeding behavior respectively, while 100% of control nymphs (n=14) performed filter feeding behavior in the immobilization experiment.

3.4 Discussion

Number of nymphs obtained on collecting trips varied seasonally and were generally higher in summer and fall dates, averaging 75 nymphs per trip. Winter and spring collecting dates produced lower numbers, averaging 25 nymphs. Very few nymphs were collected in the Red Deer River in 1994 and 1995 while many were still available in the Pembina River. It is not known why numbers dwindled so dramatically at the Red Deer River site since conditions appeared favorable for *A. neavei.*

The technique used for rearing nymphs in the laboratory was successful. Nymphs lived up to six months at a water temperature around 6.0-8.0 C which slowed development. At least five individuals matured into sub imagoes and imagoes in captivity, an observation not reported until now. Mortality in the rearing chamber was low for cohorts from 1993 and 1994 but nymphs from 1995 did not survive as well; most individuals died after only six to eight weeks. Increased mortality may result from other experiments in the rearing chamber involving use of an insect pathogen, *Bacillus thuringiensis.* Further investigation into this possible interaction of pathogen and filter feeding mayfly nymphs may show a new, non-target species of *B. thuringiensis.*

Initial observation of behaviors in *A. neavei* nymphs was qualitative to better understand how behaviors described by earlier works fit into a broader behavioral context. Behaviors described here (see 3.3.2) agree with earlier observations of Clifford and Barton

(1979) on nymphs under non-flow conditions and for the observations made by Soluk and Craig (1988) for nymphs subjected to flow. Approximately 50 nymphs were observed to determine their stereotypic behaviors. While the descriptions are not a complete ethogram, they help to answer the question of how nymphs of *A. neavei* detect water flow. Observation of nymphs in water clean of any visual cues (*i.e.* no organic particles in water column or substrate) showed that vision is not a trigger for filter feeding behavior since nymphs fed in both clean and dirty water (see 3.3.1). These results helped to narrow down where sensory information is perceived about water motion, primarily the antennae.

The second set of 25 nymphs were observed in the small flume and videotaped to determine the time period of each behavior. Eleven were not used in the final analysis because of poor visibility, damage to nymphs or because they were in a pharate stage. When pharate, a nymph is ready to shed its exuvae and emerge to the next instar (Gillot 1980, Chapman 1982). Such nymphs did not feed but maintained their position in the substrate in preparation for emergence and occasionally performed pit maintenance behavior.

The first behavior described was termed settle down behavior (see 3.3.2.1). Consistent with the description by Soluk and Craig (1988), a nymph buries itself in the substrate with only the head and antennae exposed to water. It then excavates a pit in front of its mouth parts, while simultaneously undulating its abdomen were observed, which moved water past the gills for respiratory purposes. Settle down behavior, observed in all nymphs and videotaped,

helped to determine if any experimental stress affected the nymphs' ability to detect water velocity.

The next behavior, termed collector-gatherer (see 3.3.2.2), was observed in nymphs under non-flow conditions as described by Clifford and Barton (1979), Merrit and Cummins (1978) and Edmunds *et al.* (1976) and in those exposed to low-flow (under 10.0 cm/s) conditions. If nymphs were left for long periods of time in still or low-flow water, a series of furrows appeared in the substrate, caused by nymphs actively plowing through the substrate, presumably in search of new food. This behavior was not observed under faster flow. These changes in behavior suggest that *A. neavei* is an opportunistic feeder and once water velocity increased beyond 10.0 cm/s, nymphs started a different feeding behavior (Soluk and Craig 1988). This behavior was termed filter feeding (see 3.3.2.3).

Mean settle down and latency times to filter feeding behavior were approximately doubled to 300 seconds to cover the range of variability. As seen in Figure 3-7, means for both settle down and latency times are close. However, the range observed for latency time was much larger than for settle down time, some nymphs commenced feeding quickly and some very slowly after velocity changes. Source of variability is not known, but all behaviors were stereotypic. High variability is known from other studies of aquatic insect feeding behaviors (Chance 1970, Devitt and Smith 1985, McShaffrey and McCafferty 1986).

Results from the ablation experiment showed significant involvement of antennae in detection of water velocity. Comparing mean settle down times of the control to treatments (Fig. 3-8)

showed a difference among groups. This indicated some stress on nymphs with ablated antennae. Further analysis of data showed a difference between 0.3AB treatment and control (Table 3-1) and not between other groups. This may be due to small sample size.

Filter feeding only occurred in 0.3AB nymphs at 18.0 cm/s and not in 0.6AB and 1AB nymphs, except for one nymph in 1AB which filter fed at velocities of 0.0, 12.0 and 18.0 cm/s. This nymph started filter feeding when the velocity changed to 12.0 cm/s and continued to do so at velocities of 18.0 cm/s and 0.0 cm/s. It seemed to be permanently in the filter feeding mode rather than inhibited like other treated nymphs. Absence of filter feeding in other nymphs indicated that loss of flagella reduced effectiveness of the antennae as velocity detecting organs.

Filter feeding occurred as expected for control nymphs at both 12.0 and 18.0 cm/s. It was expected that 0.3AB treatment would feed at 12.0 cm/s and the 0.6AB treatment at 18.0 cm/s to produce a latency curve, but removal of the flagella at these lengths reduced sensitivity to water movement, more than was expected. Loss of surface area of the flagella, therefore, reduced the amount a flagellum moved about in the pedicellar joint and, did not excite enough sensilla to trigger filter feeding.

Comparing mean latency times of control and 0.3AB nymphs showed a difference between them (Fig. 3-9). This indicated that, although a larger stimulus was needed to trigger the filter feeding behavior in 0.3AB nymphs, it occurred much faster than in the control. Decreased latency time to feeding has not been reported for

ablatory experiments on other aquatic (Hughes 1958), or terrestrial insects (Heran 1959, Gewecke 1970, Linsenmair 1970).

Since ablation experiment inhibited velocity detection by shortened antennae, immobilization of antennae was done. This experiment confirmed that loss of velocity detection was due to reduction of flagella mobility and not experimental stress associated with the ablation experiment. Isolation of the pedicelar-flagellar joint also determined that sensilla located in the pedicel detect velocity. Both control and treated nymphs settled down within 300 seconds of introduction, similar to nymphs from the ablation experiment.

Filter feeding behavior was evident for nymphs from the control and treatments of G2F and G1F. When at least one flagellum was free to move with water flow at the pedicelar-flagellar joint, *A. neavei* nymphs detected water velocity. Nymphs with both pedicelar-flagellar joints immobilized did not filter feed and appeared not to detect water velocity. Mean latency times for the G2F and G1F treatments showed no significant difference from the control (Fig. 3-10). This suggested that glued antennae was less stressful to nymphs than ablation.

Results of the immobilization experiment substantiated those of the ablation experiment which showed antennae are critical structures in detecting water velocity. This experiment also demonstrated which parts of the antennae are important for velocity detection. When glue immobilized flagella at the pedicellar-flagellar joint of both antennae, no filter feeding was observed. However, if just one flagellum was free to move with water flow, filter feeding

behavior was evident for most nymphs (Fig. 3-10). Isolation of the pedicelar-flagellar joint provided strong evidence that sensilla in the pedicel are important in detecting velocity.

This evidence is consistent with results of other studies on insect antennae where sensitivity to motion of flagella caused by a moving medium (Tischner 1953, Burkhardt and Schneider 1957, Burkhardt 1959, Heran 1959, Burkhardt and Gewecke 1966). Burkhardt and Gewecke (1966) showed using functional morphology and electrophysiological studies on blow flies, that their antennae are used to detect motion. Gewecke (1974) showed in grasshoppers that a flagella moved at the pedicelar joint and detected air currents. Isolation of one antenna by Hughes (1958) showed that an insect can use a single functional antenna to detect motion.

An unexpected result of my study on *A. neavei* nymphs appeared to be that behavioral differences occurred between cohorts. Cohorts consisted of nymphs from August, 1994 and October, 1995 used in ablation and immobilization experiments, and nymphs from November, 1993 used for original behavioral descriptions. Mean settle down and latency times were compared to determine any differences. Mean settle down times (Table 3-2) showed that August, 1994 and October, 1995 cohorts settled down significantly faster than nymphs of the November, 1993 cohort (Fig. 3-11). Comparison of mean latency times (Table 3-3) showed the October, 1995 cohort responded significantly faster to stimulus than either November, 1993 and August, 1994 cohorts (Fig. 3-12).

These results revealed some variability among the cohorts in response to environmental stimuli. Although the mean settle down

time for November 93 cohort was slowest, it had a shorter mean latency time to filter feeding than the August 94 cohort, whose mean settle down time was fastest of all three cohorts. Variability in behavior among cohorts may have affected response times in each experiment. Variability among cohorts and not experimental stress, may explain some of the difference for settle down times among the ablation treatments, where the 0.3AB treatment was slowest of the three treatments, which was not expected.

The percentage of nymphs filter feeding was 100% for all cohorts. The smaller sample size used from the August, 1994 cohort was due to experimental protocol. Random velocities that each nymph was exposed to, included a high velocity of 18.0 cm/s, that caused substantial shifting of substrate. Consequently, some nymphs were buried by moving sand dunes and could not be observed and excluded from analysis.

For 0.3AB nymphs, 50% fed at 18 cm/s and it is not clear why 50% did not filter feed. Perhaps ablation of antennae was too stressful for these nymphs, although Linsenmair (1970) reported a 50% reduction in sensitivity to stimuli for beetles that was not attributed to experimental stress.

Percentages of treated nymphs filter feeding in the immobilization experiment are lower than for control nymphs (Fig. 3-11). It is not known why 17% of G2F or 33% of G1F nymphs did not filter feed. These unexplained results perhaps indicate that both ablation and immobilization protocols need to be refined to reduce effects of experimental stress.

Further investigation must be done to unravel the exact function of sensilla in antennae of these nymphs and how they influence behavior. Although my results strongly indicate that antennae are the main velocity detectors, electrophysiological studies will be needed to determine exactly which of the sensilla detect which stimulus for the insect.

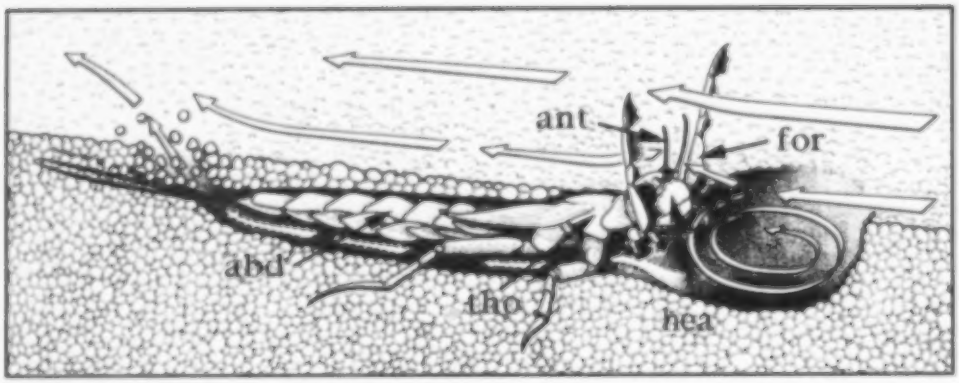

Fig. 3-1 Diagram of nymph filter feeding with forelegs (for), head (hea) and antennae (ant) extended into flow with abdomen (abd) and thorax (tho) buried under the substrate. Arrows indicate flow patterns. Modified from Soluk and Craig (1988). Not to scale.

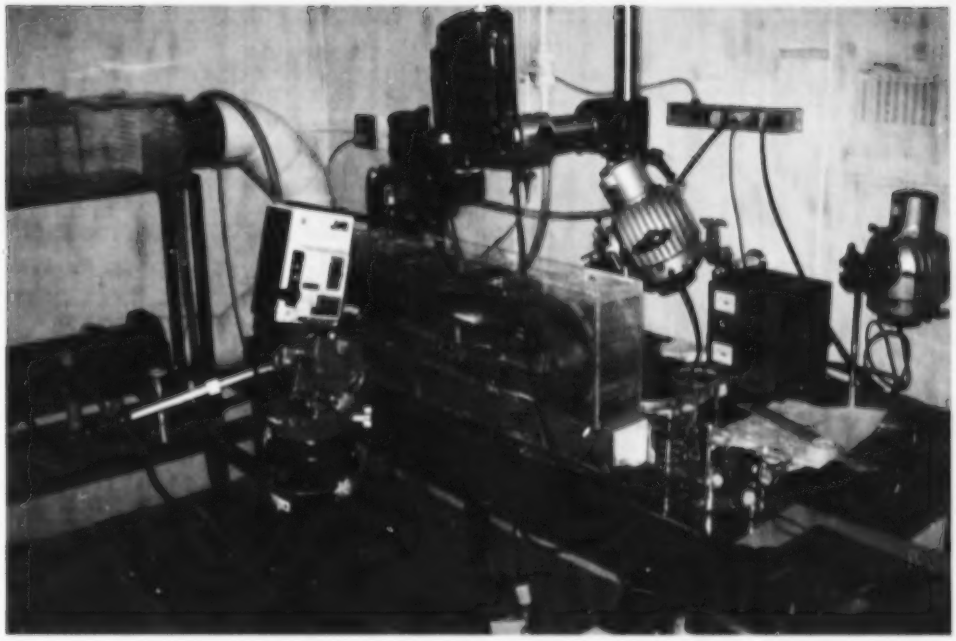

Fig. 3-2 Laboratory set up showing flume and video equipment.

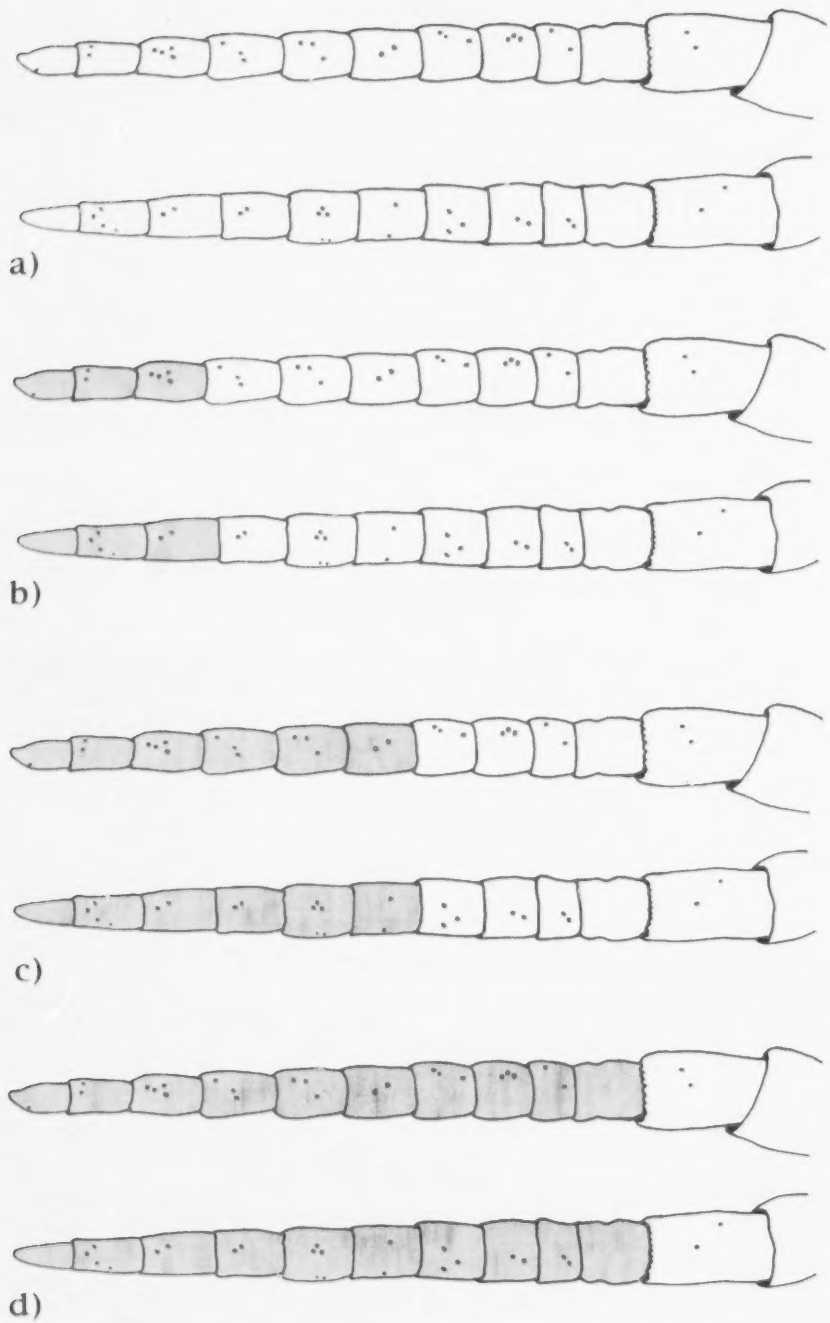

Fig. 3-3 Extent of flagellar removal in ablation experiment; a) control, b) 0.3AB, c) 0.6AB), d) 1AB. Shading indicates amount removed. Dorsal aspect, not to scale.

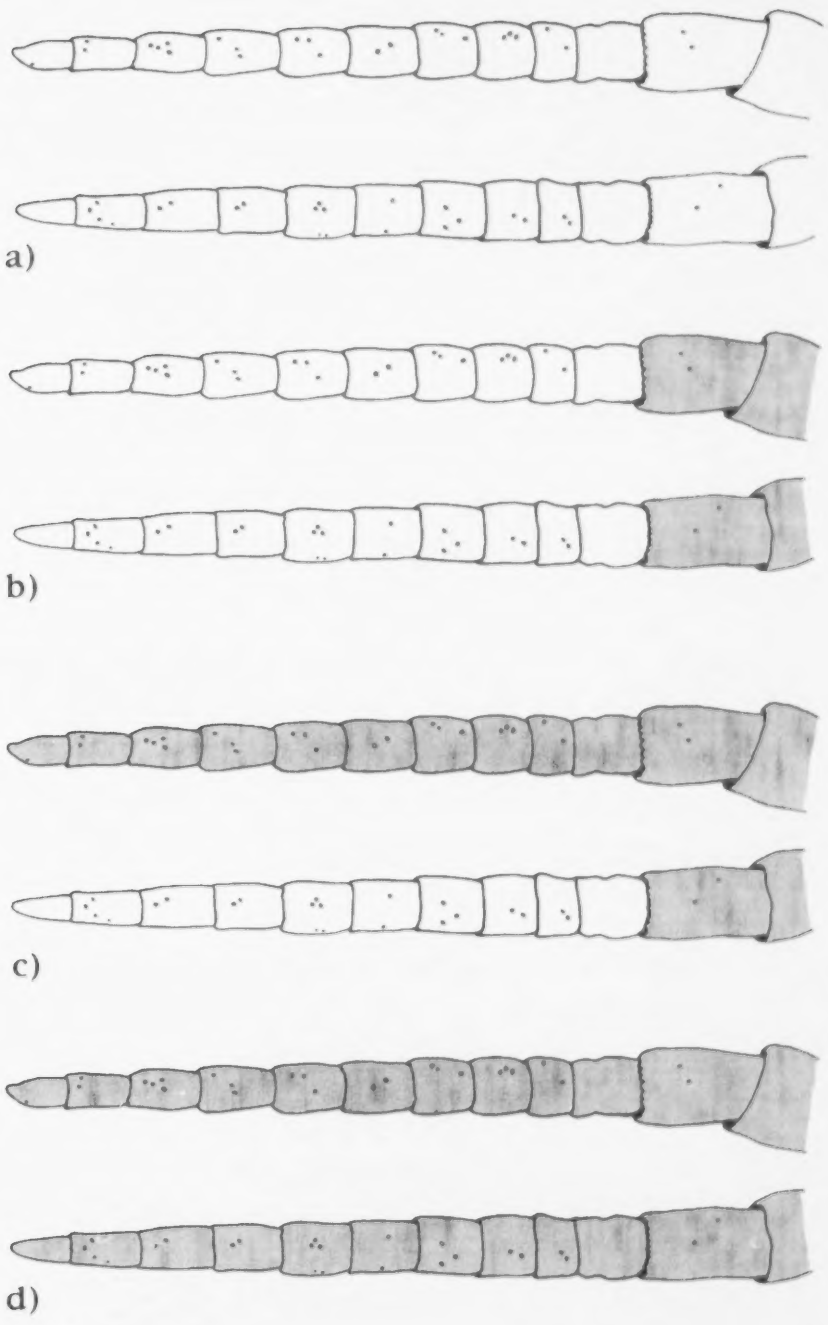

Fig. 3-4 Extent antenna glued in the immobilization experiment; a) control, b) 2 free flagella (G2F), c) 1 free flagella (G1F), d) no free flagella (G0F). Shading indicates where glue was applied. Dorsal aspect, not to scale.

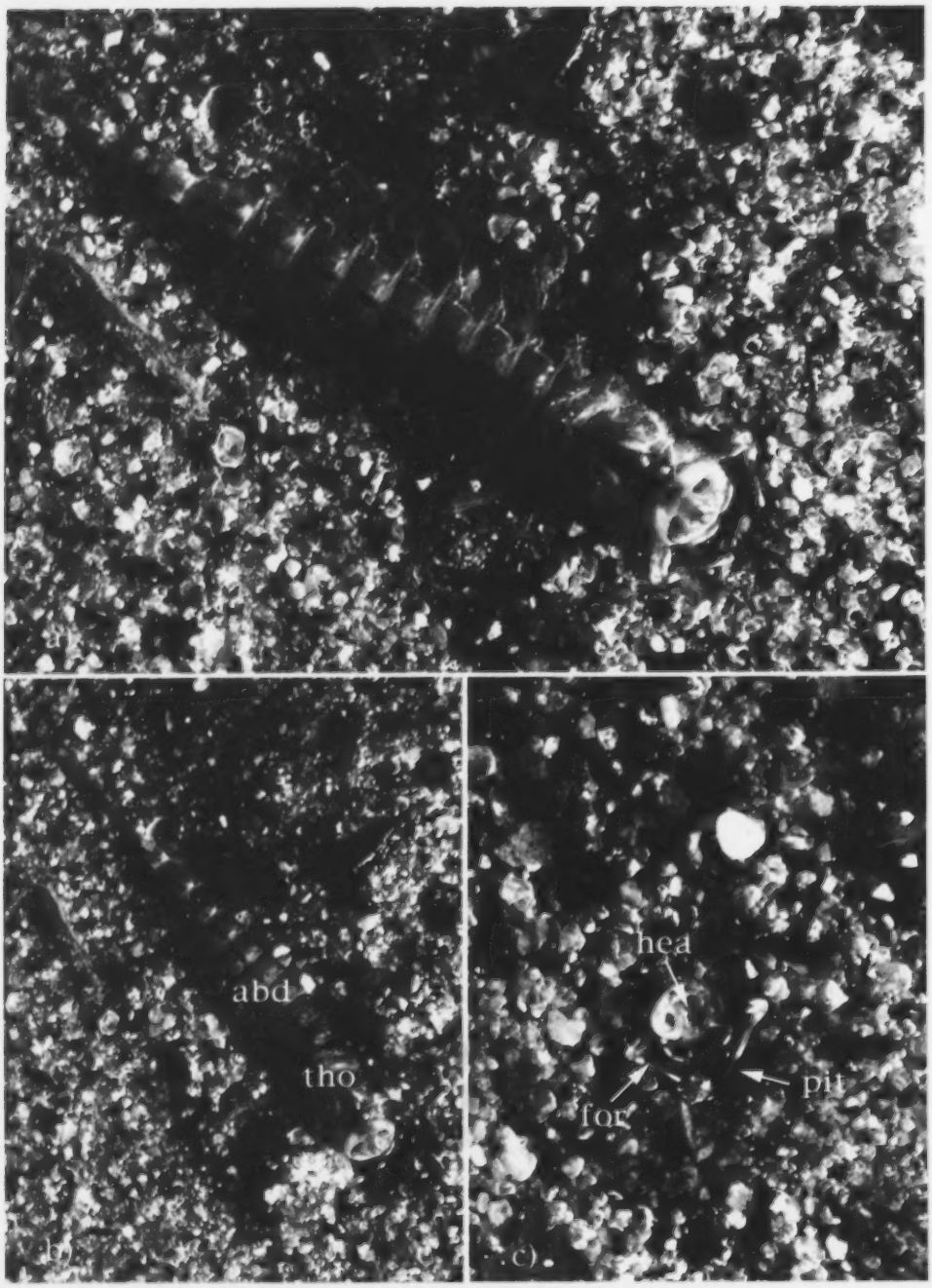

Fig. 3-5 Settle down behavior of nymphs. a) first contact with substrate, b) side-to-side motion to bury the abdomen (abd) and thorax (tho), c) final excavation of pit (pit) by forelegs (for) leaving head (hea) exposed.

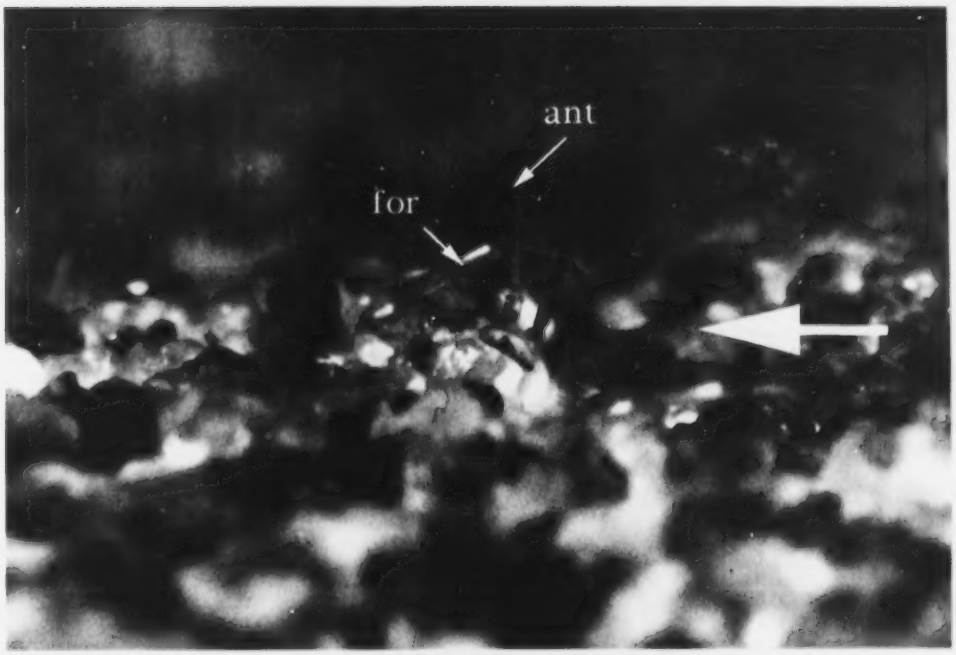

Fig. 3-6 Filter feeding behavior with forelegs (for) extended into flow. Antennae (ant) are also shown. Arrow indicates direction of flow.

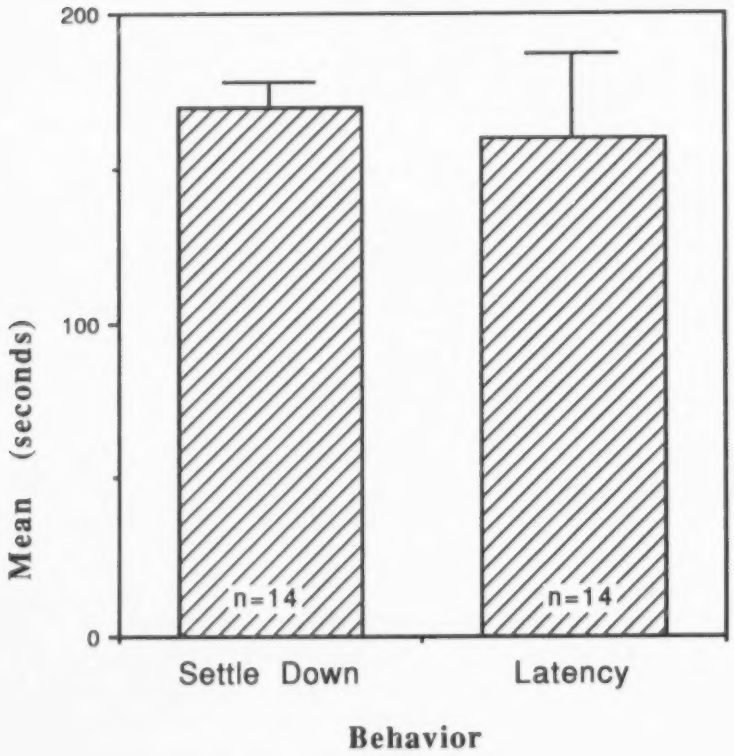

Fig. 3-7 Mean times (seconds) of behaviors for November 1993 nymphs.

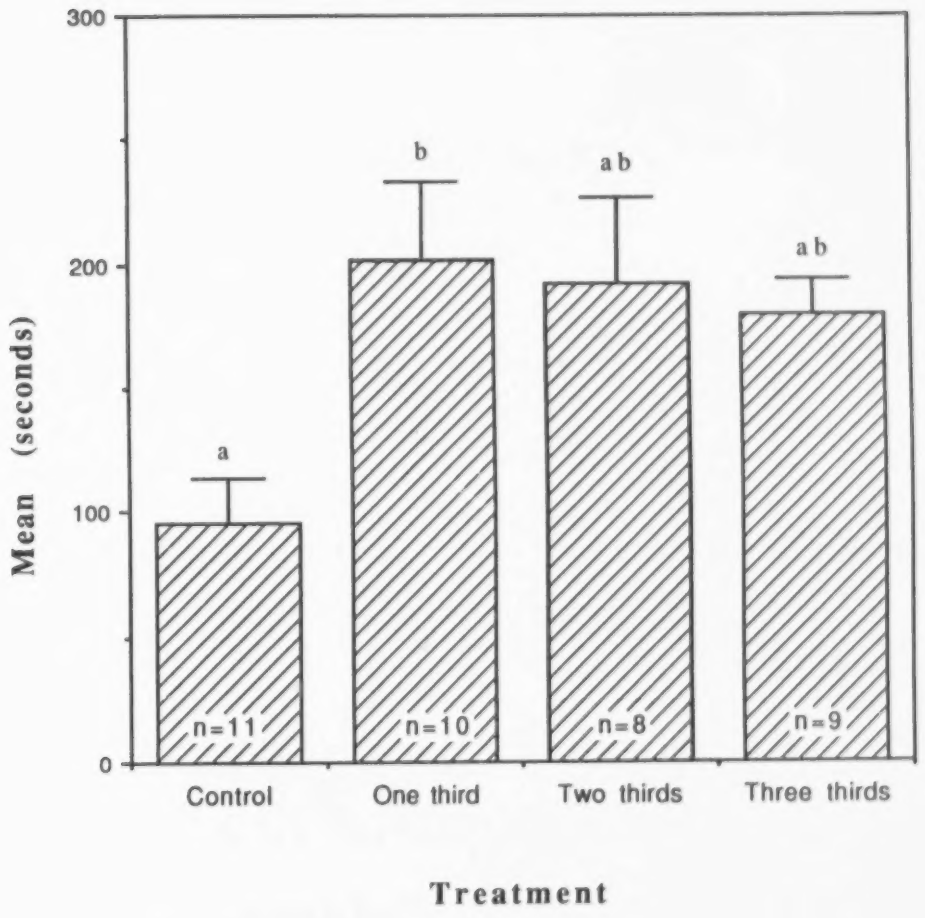

Fig. 3-8 Average time to settle down for nymphs in ablation experiment (velocity 8 cm/s). Identical letters indicate no significant difference between treatments.

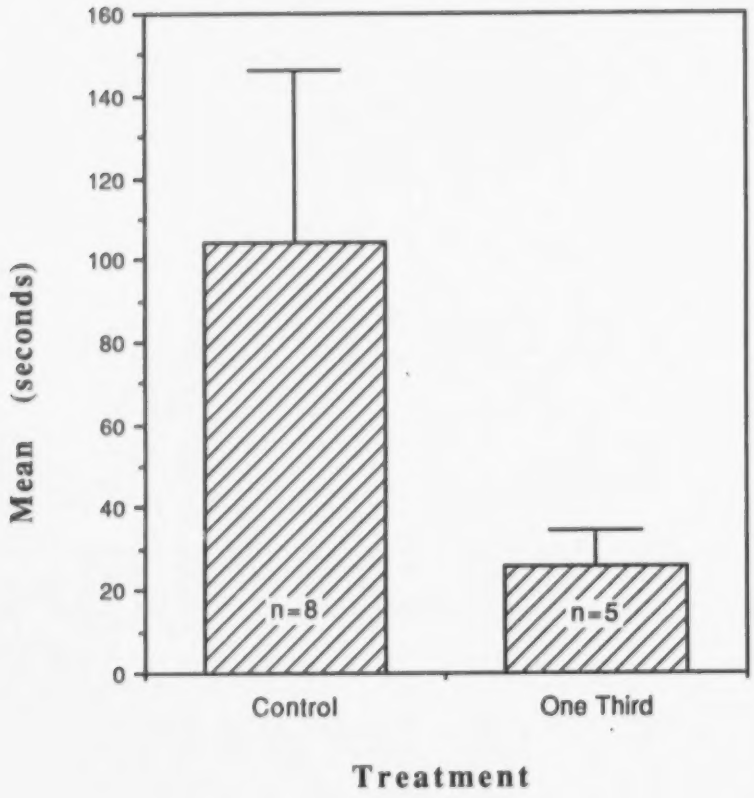

Fig. 3-9 Average latency time to filter feeding for nymphs in ablation experiment (velocity 18 cm/s).

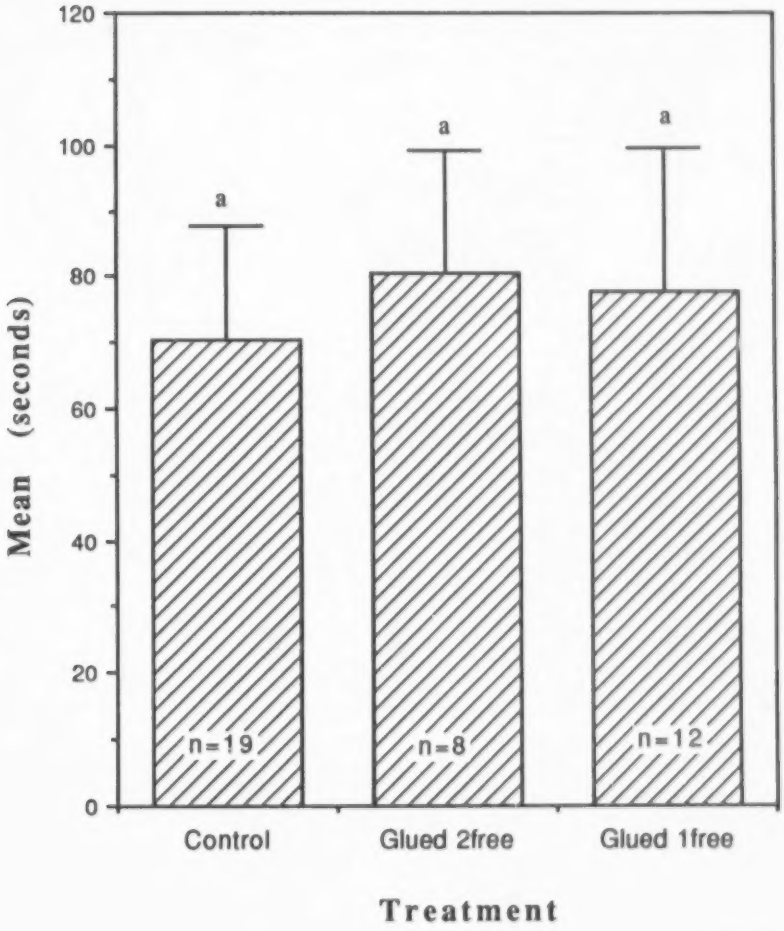

Fig. 3-10 Average latency time to filter feeding for nymphs in immobilization experiment. Identical letters indicate no significant difference between treatments.

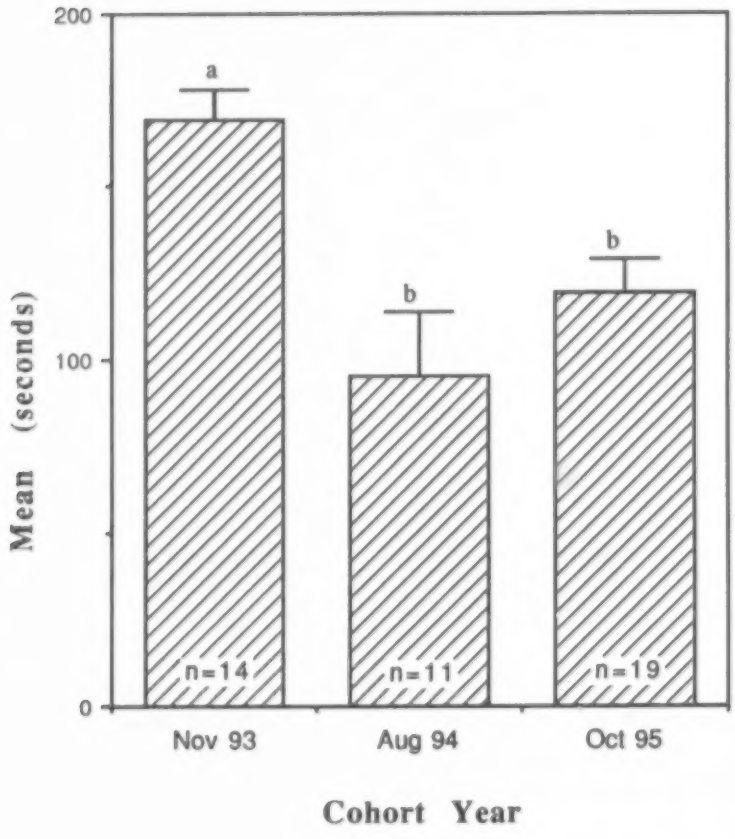

Fig. 3-11 Average settle down time per cohort year. Identical letters indicate no significant difference between treatments.

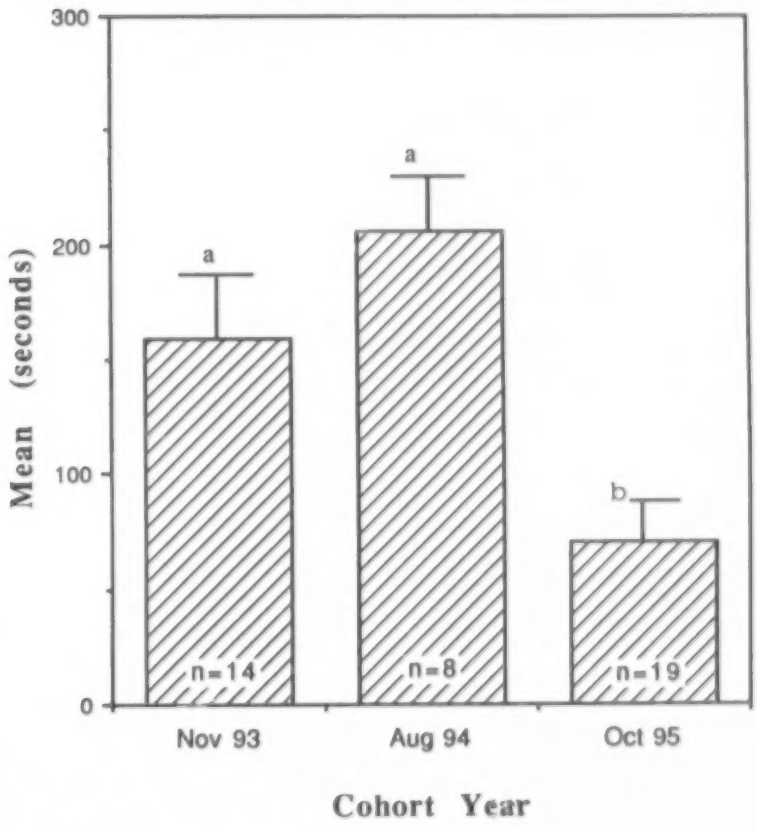

Fig. 3-12 Average latency time to filter feeding per cohort year. Identical letters indicate no significant difference between treatments.

B vs A Comparison	Difference RB - RA	SE	Q	Q 0.05, 4
1 vs2	13.63	4.85	2.81	2.639
1 vs 3	12.54	5.16	2.43	2.639
1 vs 4	12.67	4.99	2.54	2.639
3 vs 2	1.09	5.27	0.21	2.639
3 vs 4	0.13	5.4	0.024	2.639
4 vs 2	0.96	5.1	0.19	2.639
1=control	2=0.3AB	3=0.6AB	4=1AB	

Table 3-1 Nonparametric multiple comparison test for settle down time in ablation experiment.

B vs A Comparison	Difference RB - RA	SE	Q	Q 0.05, 3
1 vs 2	19.334	5.174	3.737	2.394
1 vs 3	12.578	4.523	2.781	2.394
3 vs 2	6.756	4.866	1.388	2.394
1=Nov 93	2=Aug 94	3= Oct 95		

Table 3-2 Nonparametric multiple comparison test for settle down time per cohort year.

B vs A Comparison	Difference RB - RA	SE	Q	Q 0.05, 3
1 vs 2	5.607	5.309	1.056	2.394
1 vs 3	16.908	5.049	3.349	2.394
2 vs 3	11.301	4.219	2.679	2.394
1=Nov 93	2=Aug 94	3=Oct 95		

Table 3-3 Nonparametric multiple comparison test for latency time per cohort year.

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4. General Conclusions

Results of this study has show that nymphs of *Ametropus neavei* are able to detect water velocity using their antennae. Description of the antennae showed that the necessary internal and external sensilla (Debauche 1935, Dethier 1963, Schneider 1964, Chapman 1982, McIver 1985, Zacharuk 1985) to be present to detect movement of antennae caused by water flow.

Observations of feeding behavior showed that *A. neavei*, is a collector-gatherer (Merrit and Cummins 1978, Clifford and Barton 1979) or a filter feeder (Soluk and Craig 1988), depending on flow conditions. Nymphs behaved as collector-gatherers in non-flow and low-flow conditions and as filter feeders under high-flow conditions (> 10.0 cm/s), nymphs behaved . This change in feeding behavior, coinciding with changes in water velocity, suggested that nymphs are able to detect changes in water velocity.

Perception of flow changes was assumed to be via the antennae of the nymphs, since only head and antennae are exposed to water flow in buried nymphs. Observations of antennae in different flow conditions revealed that they were moved by water flow. Once water velocity increased beyond a threshold of 10.0 cm/s, it was assumed enough antennal sensilla were excited by increased movement and induced changes in feeding behavior.

Experimental evidence demonstrates that antennae are velocity detectors for nymphs. When antennae were modified either by flagellar ablation or immobilization, no behavioral changes were

observed when water velocity changed. Lack of movement by flagella at the pedicellar-flagellar joint, inhibited the antennae from functioning as velocity detectors.

All other behaviors examined in experimentally modified nymphs were within parameters observed for control nymphs. Modified nymphs behaved as if they were in non-flow conditions, because they could not detect change in water velocity. This provided strong evidence that mechanoreceptors in and on the pedicel are used to detect flagellar motion caused by water flow. This is consistent with results of work done on equivalent sensilla located on antennae of other insects (Hollick 1940, Roth 1948, Heran 1956, Bässler 1958, Burkhardt and Gewecke 1966, Linsenmair 1970, Gewecke 1974, McVean 1991).

Nymphs of *A. neavei* are an opportunistic feeder rather than their original collector-gatherer classification by Merrit and Cummins (1978). Ability of a nymphs to change from collector-gatherer to filter feeder and back, frees them from dependence on only one food resource. *A. neavei* nymphs are capable of removing organic matter from particles and in filtering material suspended in the water column. Similar opportunistic behavior has been reported in nymphs of *Stenacron interpunctatum* (McShaffrey and McCafferty 1986), *Rhithrogena pellucida* (McShaffrey and McCafferty 1988) and *Ephemeralla needhami* (McShaffrey and McCafferty 1990).

Although nymphs of *A. neavei* are highly adaptable, they are not wide spread in lotic systems. Rather, they occur exclusively in areas of shifting sand in large rivers (Allen and Edmunds, Jr. 1976, Clifford and Barton 1979, Soluk and Craig 1988). They may prefer

such habitat because there is little competition for resources (Soluk 1983) since few other invertebrates occur there. Filter feeding is theoretically more efficient than a collector-gathering in rivers with these flow conditions (Rubenstein and Koehl 1977).

Examining factors affecting organisms in lotic systems is important in understanding ecological relationships of a species in question. Study of behavior and functional morphology is crucial to understanding these relationships prior to assumptions made about the ecological roles that different species have in lotic systems (McShaffrey and McCafferty 1986).

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